

THE BOTANICAL GAZETTE

EDITOR
E. J. KRAUS

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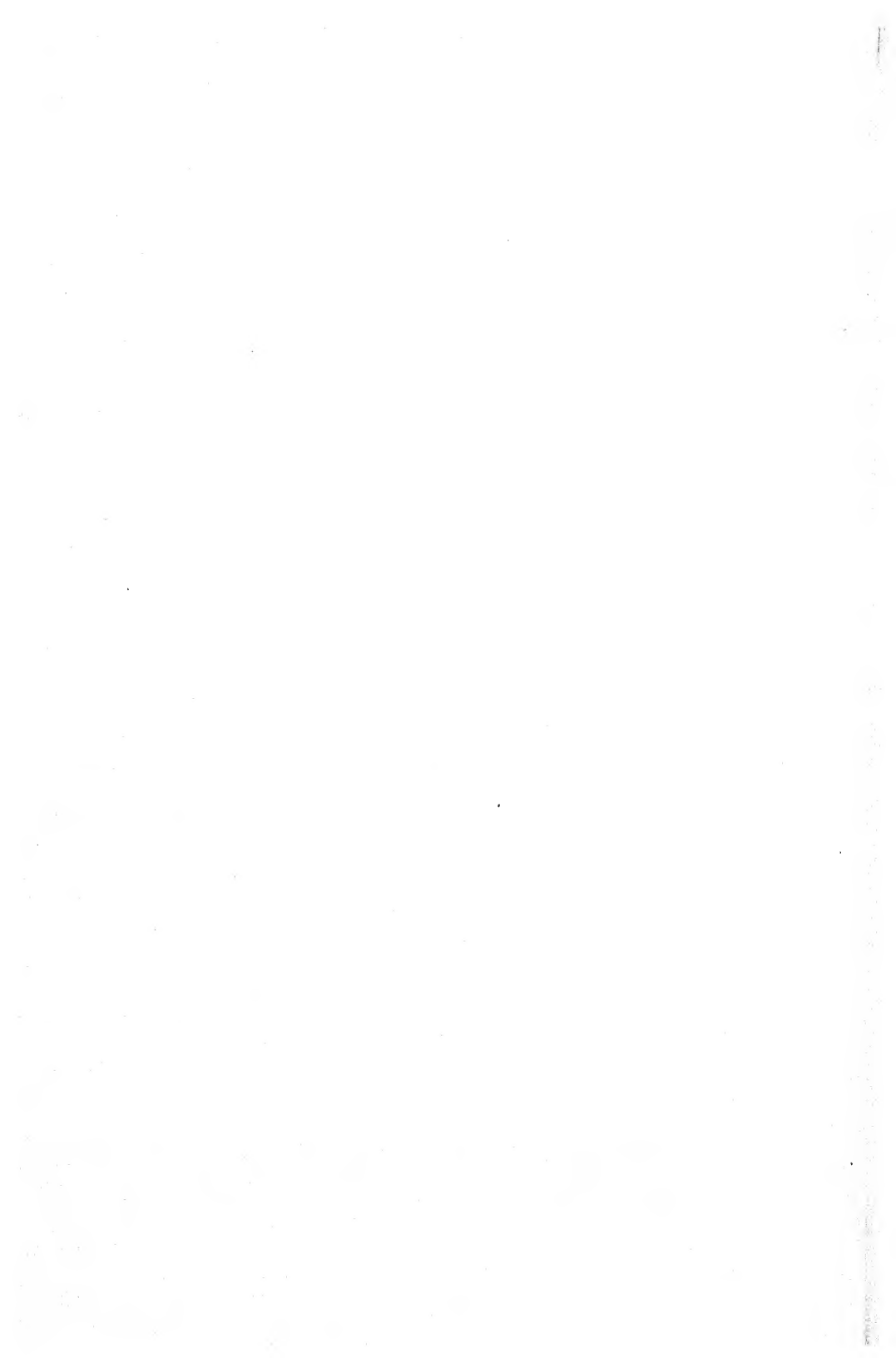
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THE BOTANICAL GAZETTE

September 1941

EMBRYOGENY OF THE PODOCARPACEAE

J. T. BUCHHOLZ

(WITH FIFTY-TWO FIGURES)

Introduction

The genus *Podocarpus* is divided, according to PILGER (20), into two subgenera—*Stachycarpus* and *Protopodocarpus*. The latter subgenus is further subdivided into several sections: *Dacrycarpus*, *Microcarpus*, *Nageia*, and *Eupodocarpus*. Several years ago the writer (4) contributed a series of stages in the embryogeny of several species of *Stachycarpus*. The embryogeny of *Dacrydium cupressinum* had been described earlier (3).

The present paper is concerned chiefly with the embryogeny of *Dacrycarpus*, *Nageia*, and *Eupodocarpus*. The embryogeny of additional representatives of the subgenus *Stachycarpus* and of *Phyllocladus* is described more fully than formerly, with special reference to details concerning the binucleate embryonic and apical cells. This paper records practically all the properly preserved embryological material which it has thus far been possible to obtain from conservatories in this country, from correspondents in the Southern Hemisphere, or from collections made by American botanists while abroad. I am indebted to Mr. H. W. Lawton of Wellington, New Zealand, for collections of *Podocarpus totarra* and several other species; to Dr. E. W. Sinnott for *P. dacrydioides*; and to Dr. M. S. Markle for specimens of *P. urbanii* obtained on Blue Mountain, Jamaica. The material of *P. macrophyllus maki* (*P. chinensis*) was collected by the writer from the conservatory of the New York Botanical Garden. While I have observed many species of the Podocarpaceae on estates and in parks and public gardens in California, very few of the species grown in this country, aside from *P. macrophyllus*, were found to produce seeds. Some in California produce pollen cones and ovules, but unfortunately many of the plantings of rare species are so scattered as isolated specimens that

the dioecious species lack the facilities for pollination. Some of the material of *Phyllocladus alpinus*, used previously by YOUNG (27), was found to include a number of stages in the embryogeny. With the exception of the latter, which had been sectioned, and some of the material of *P. totarra*, which was also cut serially, the material was dissected and stained following the methods described elsewhere (5).

The material of the Nageia section of *Podocarpus* and that of *P. gracilior* and *P. amarus*, belonging to the *Stachycarpus* subgenus, as well as that of a few South American podocarps, was all obtained from dried herbarium specimens. Fully enlarged ovules and seeds were cracked open so that the female gametophyte or endosperm could be removed and placed in water for a day or two. In many instances this tissue would swell by imbibition to its former size and shape, regaining its full turgor. The technique of dissection which has been described (5) for living or preserved material could then be used. In the instances in which the female gametophytes would not swell to their former size and shape, dissections were much more difficult but not impossible.

Fully enlarged ovules may be in stages before fertilization or have fully matured embryos. Only an occasional ovule will yield embryos in desirable early stages of development. As might be expected, the embryos obtained from dried material are usually somewhat shriveled and deformed. Usually considerable endosperm tissue adheres to the surface of the suspensor and embryonic tips, and this is difficult to remove. These tissues interfere somewhat with microscopic observation, and their complete removal is likely to damage parts of the embryonic structures. Nevertheless, after imbibition followed by staining with phloxine, the cell arrangement and number may be easily ascertained, and sometimes the nuclei may be observed; even chromosomes in late prophases of nuclear division may occasionally be recognized.

For the use of herbarium specimens, I am indebted to Dr. E. D. Merrill, Director of the Arnold Arboretum; Dr. Herbert L. Mason, Curator of the University of California Herbarium; Drs. Paul Standley and Julian Steyermark, Field Museum of Natural History; and Dr. J. M. Greenman, of the Missouri Botanical Garden.

The following list gives the species whose embryogenies are discussed or described here or previously, with some of the synonymy, following PILGER (19, 20) and in conformity with the 1930 and 1935 Amendments to the International Rules of Botanical Nomenclature.

Dacrydium cupressinum Sol. ex Forster New Zealand (3, 22)

Podocarpus L'Herit. ex Pers.

Subgenus: *Stachycarpus* Engl.

P. spicatus R. Br. ex Bennett New Zealand (4, 22)

P. ferrugineus D. Don New Zealand (4, 22)

P. usambarensis Pilger East Africa; cultivated in Jamaica (4)

P. gracilior Pilger Mountains of British East Africa; Uganda; Abyssinia

P. amarus Blume Northeast Australia; Java; Sumatra; Philippine Islands; New Guinea

Subgenus: *Protopodocarpus* Engl.

Section I *Dacrycarpus* Endl.

P. dacrydioides Rich. New Zealand (22)

P. imbricatus Blume British North Borneo and monsoon region

Section II *Microcarpus* Pilger (no material obtained)

Section III *Nageia* Endl.

P. nankoensis Hayata Formosa and Hainan

P. nagi (Thunb.) Zoll. & Moritz Southern Japan; China (26)

P. blumei Endl. Sumatra; Philippine Islands

Section IV *Eupodocarpus* Endl.

P. urbanii Pilger Jamaica

(*P. coriacea* Hook.) The Jamaica plant *P. coriacea* Hook. = *P. urbanii*, not *P. coriaceus* L. C. Rich.; probably not the plant collected by COKER (8)

P. macrophyllus (Thunb.) D. Don in Lambert . . . Japan (23, 26); cultivated in California

P. macrophyllus maki Endl. Japan; cultivated in New Orleans and California; North Carolina to Florida

The plant collected by COKER (8) at Darlington, North Carolina, probably belongs here

(*P. chinensis* Wall. ex Endl.). This species, according to KÄMPFER, is often cultivated in conservatories of botanical gardens) conservatory of New York Botanical Garden

P. glomeratus Don Ecuador

P. coriaceus L. C. Rich. Venezuela; Puerto Rico

P. purdeanus Hook. Jamaica

P. mutudai Lundell Southern Mexico

P. totarra A. Cunn. (*P. totara* D. Don) New Zealand (22)

P. hallii T. Kirk New Zealand (22)

(*P. totarra* var. *hallii* [T. Kirk] Pilger)

P. nivalis Hook. New Zealand (22)

Phyllocladus alpinus Hook. f. New Zealand (1, 13, 14, 27)

Saxegothaca conspicua Lindl. Chile (10, 17); cultivated in British Isles

In the descriptions which follow, the *Nageia* group of *Podocarpus* is treated out of its order, since it is more convenient to describe this embryonic type last, together with several additional members of the *Stachycarpus* subgenus. This plan of treatment permits rendering the descriptions based on some of the dried ma-

terial by comparison with the figures obtained from properly fixed material. This order also avoids the necessity of including a larger number of illustrations.

Investigation

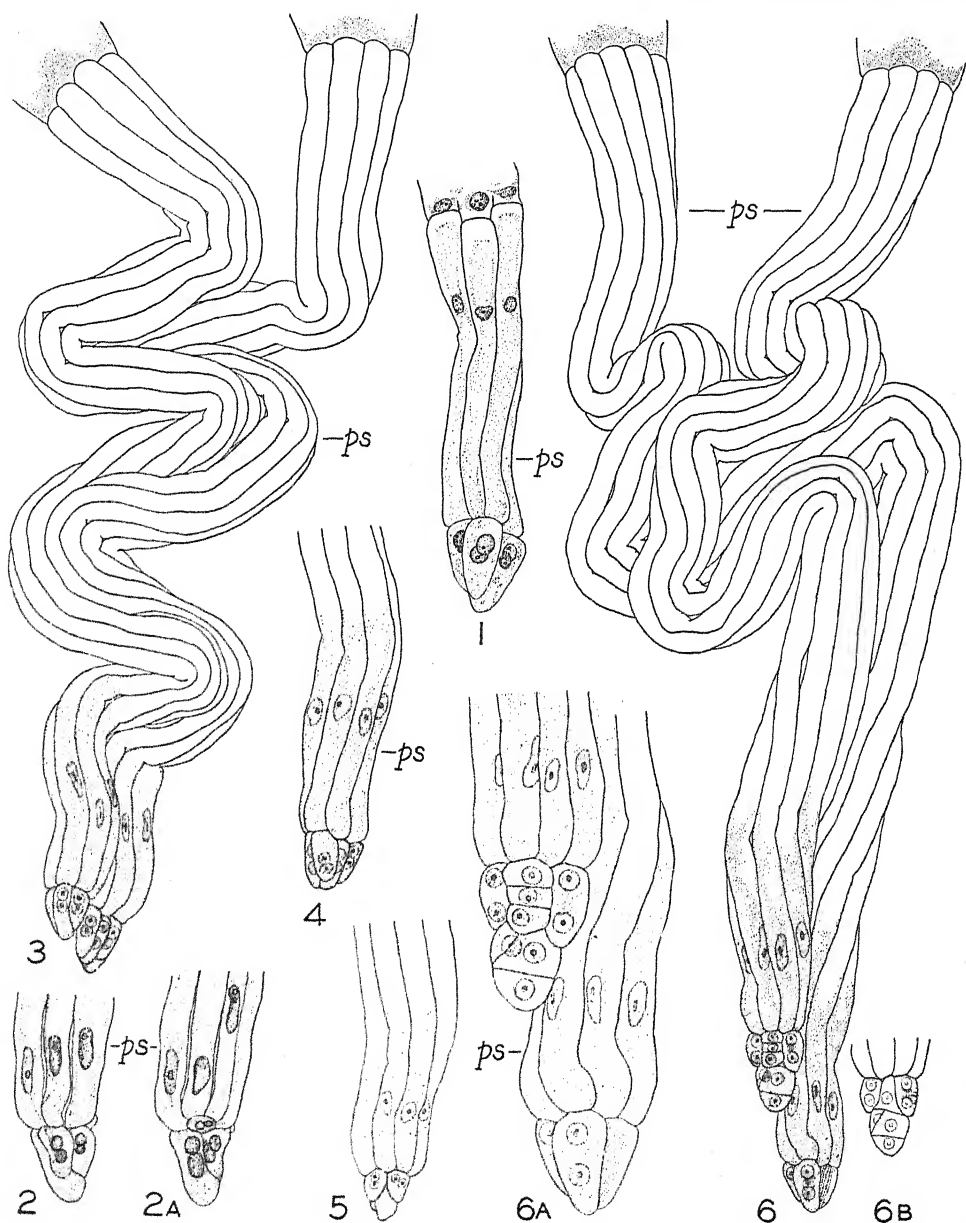
PROTOPODOCARPUS, SECTION DACRYCARPUS

PODOCARPUS DACRYDIOIDES.—The accompanying illustrations show a number of stages in the development of suspensor and early embryo of *P. dacrydioides*. These were dissected from preserved alcoholic material killed at Croydon, New Zealand, about January 30. While SINNOTT (22) covered many of the stages in the reproductive structures, including figures showing the sperm and egg nuclei side by side in fertilization, the proembryo of *P. dacrydioides* beyond the first division of the zygote was not included. The earliest stage obtainable from dissected material is shown in figure 1, in which the prosuspensor has begun to elongate but in which a tier of relict nuclei is still found above it. The embryo system shown in this figure has seven or more elongating cells in the prosuspensor, bearing four binucleate cells below at the tip. Figure 2 is from an embryo with a much longer prosuspensor, made up of nine cells, which bears five binucleate cells at the tip; figure 2, a surface view, shows only three of the embryo initials, while figure 2a of the same embryo group in the lower plane of focus reveals two other binucleate cells, one of which is located in an unusual position between the prosuspensor cells and the others. The binucleate cells are probably organized in the proembryo and may be regarded as actual or potential embryo initial cells.

In both of these embryo systems one of the embryo initials is much larger than the others. The largest may be located either at one side, as in figure 1, or more nearly in the center of the group. In early stages all binucleate cells are in contact with the prosuspensor at some point; that is, they usually form part of a single tier.

Figure 3 shows two neighboring embryo systems with prosuspenders twisted in the characteristic manner, the one at the left with three embryo initials and the one at the right with four. By the time this stage is reached the groups of embryonic initials are deeply imbedded in the female gametophyte, but they still remain as binucleate cells. In figure 4, the lower end of a prosuspensor composed of about ten elongated cells bearing five embryonic units is shown. In figure 5, the largest one of the group of five embryo initials has become aborted. Its disintegrating contents stain more deeply and traces of the nuclei are no longer observable.

Figure 1 shows the free nuclei still in the base of the egg above the prosuspensor, forming a tier of relict nuclei, while figures 3 and 6, showing the upper ends of several prosuspenders, indicate a complete absence of rosettes. Rosette cells could



FIGS. 1-6.—*Podocarpus ducrydioides*: Fig. 1, earliest observed stage showing tier of relict nuclei above prosuspensor (ps) bearing four binucleate cells; $\times 150$. Fig. 2, surface view of 3 of embryonic group of binucleate cells. Fig. 2a, optical section showing cells in deeper plane of focus; $\times 120$. Fig. 3, 2 embryo systems, 3 binucleate cells borne on 9-celled prosuspensor at left, 4 or 5 units on 10-celled prosuspensor at right; $\times 120$. Fig. 4, lower part of prosuspensor having 11-12 cells above, 9 below (where it bears 4 binucleate cells); $\times 120$. Fig. 5, lower part of prosuspensor bearing 5 units with largest central unit dead; $\times 120$. Fig. 6, embryo systems, 4 binucleate cells at right, 5 embryonic units at left; $\times 120$. Fig. 6a, tips of same; $\times 240$. Fig. 6b, largest 4-celled unit still in contact with prosuspensor.

be observed only rarely in any of the embryos successfully dissected. Figure 6 represents two adjacent embryo systems in a stage slightly older than those in the preceding figure. The embryo system at the right, which may have been more deeply imbedded, shows one of the four embryonic cells aborted. The other embryo initials in this group are still binucleate. The embryo system above at the left has just reached the stage in which some of the five embryo initials are replaced by groups of walled cells. Some of the cells which remain binucleate in both embryo systems of figure 6 show faint spindle fibers between the nuclei. The binucleate stage seems to represent, as shown by the persisting spindle fibers, a long-delayed telophase. Wall formation does not take place for many days after actual separation of the nuclei. The length of this interval is shown by the amount of elongation of the prosuspensor between a proembryo—or an embryo younger than figure 1—and one of the stage shown in figure 6.

Figure 6a is a more highly magnified view of the lower end of the two systems shown in figure 6. Figure 6b shows the arrangement of the cell walls in the upper or left embryo of figure 6 as this appears in a lower focus, indicating that a terminal 4-celled embryo has now been formed, and that this largest unit, as well as each of the other embryonic units, is still in contact with the prosuspensor. One of the other embryonic units has become 3-celled, and the two on the opposite side, masked by the three which are shown, are dead. This is the oldest stage observed in *P. dacrydioides*.

In the steps which follow figure 6 it is probable that the largest embryonic unit, which becomes the more protruding terminal embryo, pushes out a suspensor which thrusts the others upward with the prosuspensor. Several of the embryos may do this, more or less simultaneously, a condition which would produce for a time a double embryo or one of several lobes, until one of the tips ultimately outgrows the others. The general situation indicates that the embryogeny of *P. dacrydioides* has cleavage polyembryony, and there is considerable resemblance in the embryogeny, thus far, to that of *Dacrydium*, after which this species of *Podocarpus* was named. A slight difference comes from the fact that in *P. dacrydioides* all embryonic units are in contact with some part of the prosuspensor, while *Dacrydium* (3) usually has two tiers of these terminal embryo initials, only one of which touches the prosuspensor.

There may be five, four, or a smaller number of embryonic units remaining by the time cell proliferation of the individual embryos is initiated. In fact, several may become aborted during the observed stages of development. Figure 6a shows that they develop independently of one another, and the probability is that the embryo which ultimately survives is derived from only a single one of the binucleate embryonic units.

The writer has characterized the type of cleavage polyembryony in *Dacrydium*

as determinate (3). That of *P. dacrydioides* seems to be similar but somewhat less definite. The successful embryo, though larger at an early stage, may not always be situated in a terminal position.

The cells of a prosuspensor do not all extend to the embryonic units below. Several may drop out, so that an embryo system such as the one shown in figure 6 may have nine or ten elongated prosuspensor cells at the upper end and only seven or eight at the lower end, where their nuclei are usually located, with the ends of the short prosuspensor cells hidden or completely inclosed by the others. Rosette cells were rarely observed. In only one embryo system out of a dozen examples was a rosette cell shown, and this had not divided.

In the 4-celled embryo of figure 6, one of its cells remains in contact with the prosuspensor, indicating that all the several binucleate units were situated in the same plane or tier below the prosuspensor. After this 4-celled embryo has greatly increased the number of its cells, a massive secondary suspensor may be expected to elongate from its distal end, pushing the smaller embryonic units and prosuspensor upward.

PODOCARPUS IMBRICATUS.—The embryogeny of *P. imbricatus* is similar to that of *P. dacrydioides*. The only material available came from herbarium specimens. Three or four binucleate cells, arranged as in figures 1-5, were observed on the end of a prosuspensor of about nine or ten cells. In a later stage a larger terminal embryo was found on the end of a massive secondary suspensor, with a few smaller embryos placed laterally at various levels along the side of this suspensor system. This arrangement indicates determinate cleavage polyembryony in a stage later than that shown in figure 6; otherwise there was nothing to indicate important variations from the preceding account for *P. dacrydioides*.

PROTOPODOCARPUS, SECTION EUPODOCARPUS

The proembryo of a member of this group of podocarp species has been described by COKER (8) and more recently by TAHARA (26). From correspondence with DR. COKER, including an exchange of specimens, it appears fairly certain that the plant whose embryogeny he described belongs to a species very close to *P. macrophyllus maki*, under which PILGER (19, 20) places as a synonym the *P. chinensis* frequently grown in conservatories. It is certain that COKER's species could not have been *P. urbanii* Pilger, under which *P. coriacea* Hook. appears as a synonym. The seeds of COKER's species are much too large and agree more closely with *P. macrophyllus maki* (*P. chinensis*) than with *P. coriaceus* Rich., which I have examined from collections obtained at Maricao, Puerto Rico, and herbarium specimens collected in Venezuela.

PODOCARPUS URBANII.—The proembryo of this species has not been observed. From the earliest observed stages in the embryo system, it must be assumed that

the proembryo is organized with two or three terminal binucleate cells borne on the end of a prosuspensor made up of 12-14 cells.

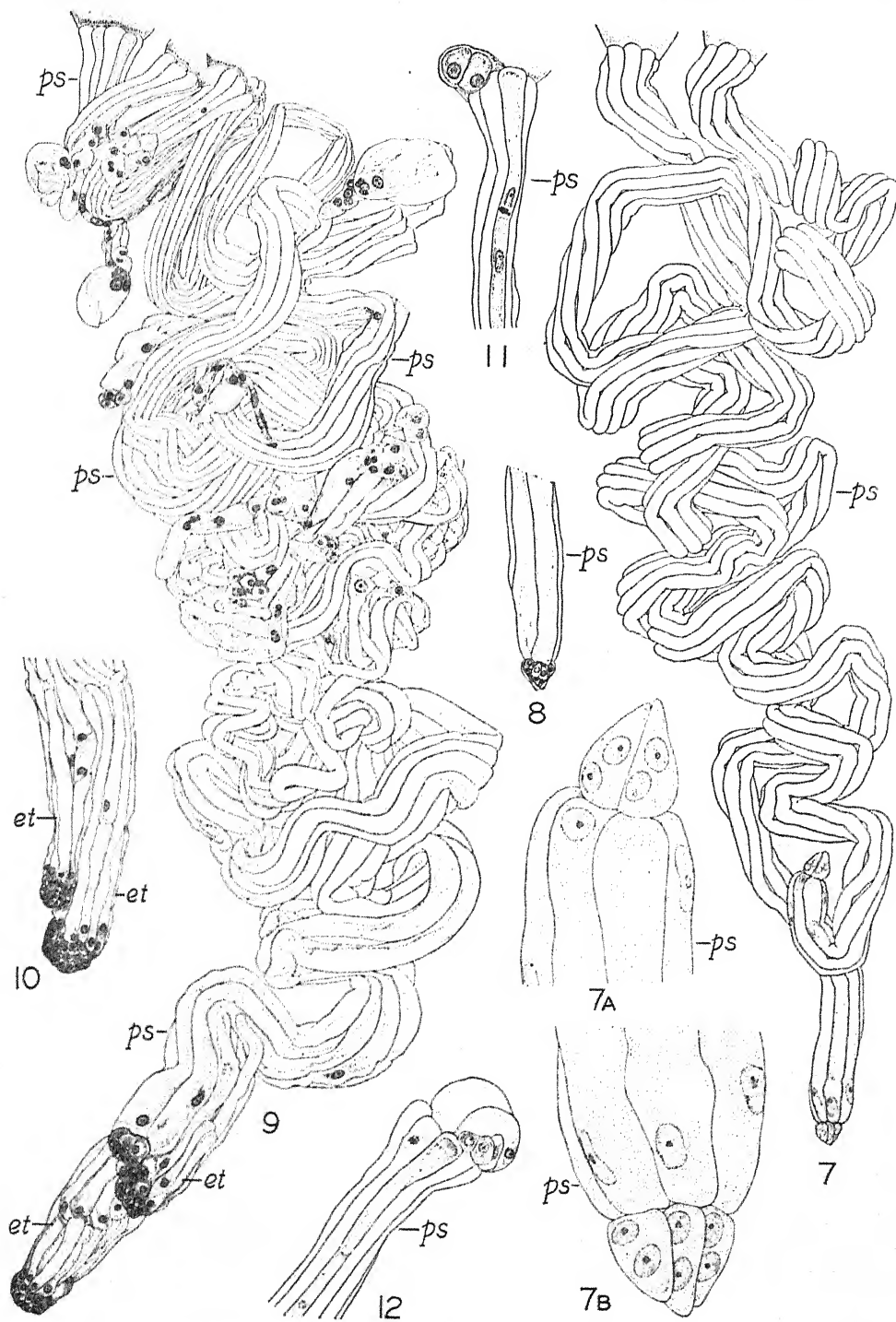
Figure 7 shows the earliest stage obtained from dissected material of *P. urbanii* collected in Jamaica. Two embryo systems are shown in which the prosuspensors have elongated greatly. The prosuspensor consists of about 12-14 cells at the top or base of the archegonium. These cells elongate together. As COKER (8) pointed out, some cells do not elongate fully, so that in figure 7 only seven or eight cells extend the entire length. The cells of the prosuspensor that drop out are frequently situated in the interior. In later stages some of these ends become free (fig. 9) and may form embryos on their ends.

There is a slight difference between *P. urbanii* and *P. macrophyllus maki* (*P. chinensis*). The former bears two or three binucleate embryonic cells (fig. 7a, b) while the latter bears only one or occasionally two. This is another feature wherein COKER's species (8) seems to agree more closely with *P. macrophyllus maki* than with *P. urbanii*. Whether the binucleate cells are two or three, they were so organized in the proembryo. There are 3-5 binucleate cells in the very early embryo of *P. dacrydioides* (fig. 1), 9-10 in *P. usambarensis* (4), and—as TAHARA has shown (26)—7 or more in *P. nagi*, where they originate from the division of nuclei in walled cells of the proembryo.

Figure 8 shows one of these embryonic tips in a later stage in which the 4-celled groups belonging to each of three units are still distinctly recognizable. A similar figure more highly magnified, with each of the three embryonic groups containing about sixteen cells, is shown in figure 18. Figures 13 and 14, also more highly magnified, show 2- and 3-lobed multicellular embryos of similar and later stages. These are still attached to the prosuspensor (though in figure 14 the largest embryo is forming a massive secondary suspensor), while in figures 10, 15, and 17, all at lower magnifications, the secondary suspensor has elongated considerably.

In the 2- and 3-lobed terminal embryos, one of the cell groups has grown faster than the others. It is likely that only a single cell group or lobe will ultimately contribute the single embryo that survives in the mature seed. Some of the individual units seem to possess an apical initial cell which persists for a brief period, at most up to the stage of an embryo of 40-60 cells. The apical cell is not easily recognized in surface views (as in these drawings) but is usually more distinct in optical section.

Figure 9 is an older stage of *P. urbanii* in which three embryo systems are found. The three terminal embryos have all become separated in this case, owing to unequal growth of their respective secondary suspensors, while the terminal embryos belonging to the other two systems have become disorganized as the ends of their prosuspensor cells have become separated. The cells of the prosuspensor that do not elongate fully may become more or less dissociated. Where their ends



FIGS. 7-12.—*Podocarpus urbanii*: Fig. 7, 2 embryo systems with prosuspensors bearing 2 and 3 binucleate cells ($\times 66$); shown in fig. 7a and fig. 7b, $\times 290$. Fig. 8, tip of similar system bearing 3 4-celled embryos. Fig. 9, 3 systems, longest with 3 embryos developing massive secondary suspensors (et), 2 other systems with terminal embryos unrecognizable and many small embryos formed on ends of prosuspensor cells; $\times 70$. Fig. 10, tip of embryo system with 2 terminal embryos on massive secondary suspensors of embryonal tubes (et); $\times 70$. Figs. 11, 12, upper ends of prosuspensors showing embryos in rosette region; $\times 140$.

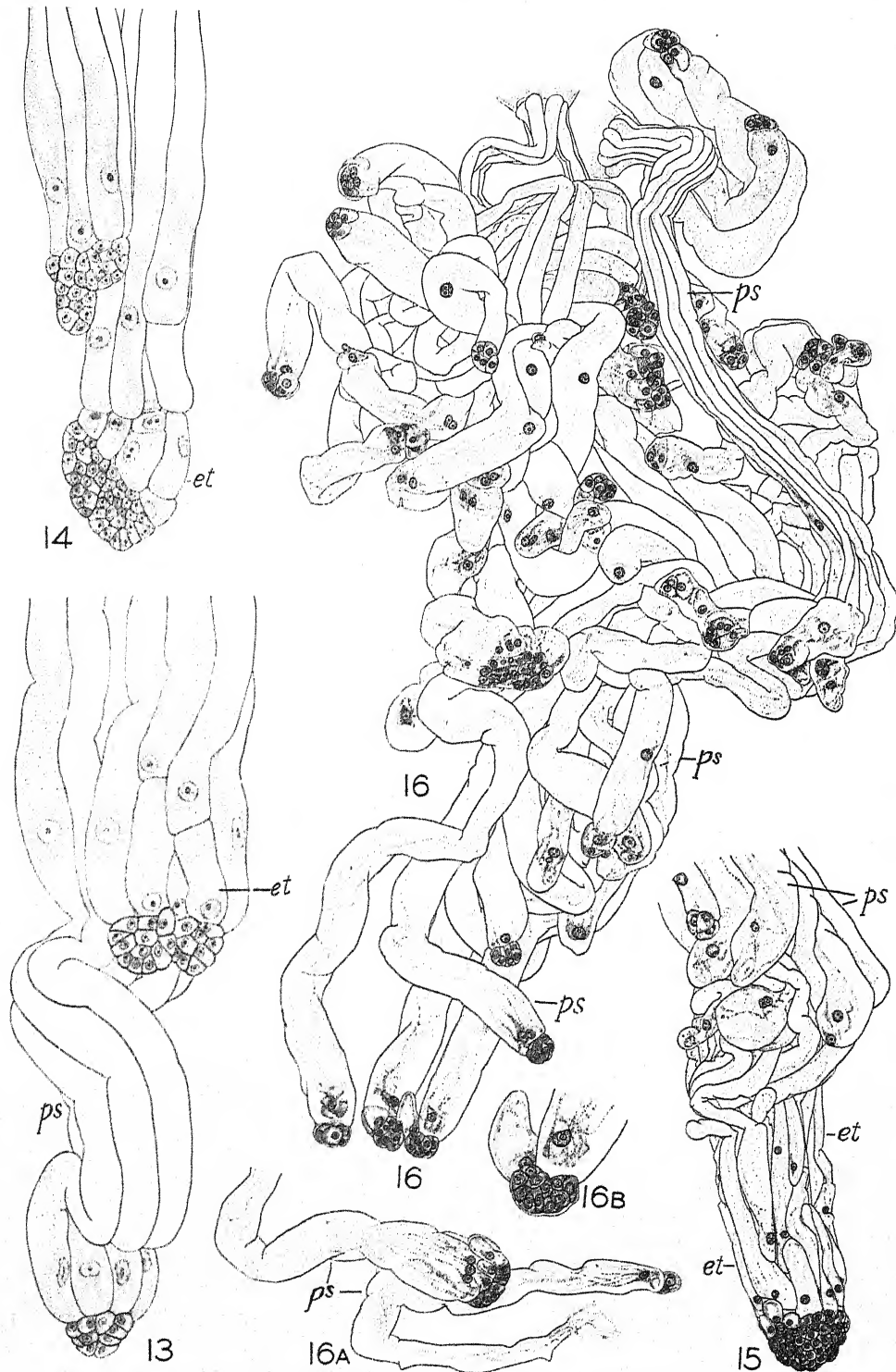
emerge from the tangle of elongated cells they may enlarge considerably and cut off at their tips one or more cells which develop into small secondary embryos. Many of them are shown in the upper half of figure 9. Figure 16 shows two systems of embryos which have disorganized into many smaller embryos, and the terminal ones shown below are not so large as in figure 9. The secondary embryos formed on the ends of prosuspensor cells therefore appear to have a primary suspensor. However, a true primary suspensor (single elongating cell given off by an embryo) is not found in this species. Thus the embryogeny of *P. urbanii* has cleavage polyembryony through the occurrence of secondary embryos formed on prosuspensor cells, as well as from the individual binucleate cells borne on the end of the prosuspensor. Some details of the manner in which the ends of prosuspensor cells form embryos will be shown in the embryogeny of *P. macrophyllus maki* which follows.

Figure 16a includes one of the largest embryos belonging to figure 16. This was broken off during dissection and may be derived from one of the terminal binucleate cells. Two others situated at the lower end of figure 16, both belonging to the system at the right side of the figure, may have come from a terminal cell. Figure 16b is an optical section at higher magnification, showing the internal arrangement of cells in relation to the apical cell. The terminal embryos belonging to the embryo system at the left of figure 16 are not recognizable with certainty, but are probably included among the many embryos in the upper half of this figure.

A total of twenty-six or more embryos is shown in figure 16. There are still more, but a number of them are situated beneath other structures. There are more than thirty derived from two fertilized eggs. The number of embryos of all kinds is therefore about fifteen per system, or nearly one for each prosuspensor cell plus two or three derived from the terminal binucleate cells shown in figure 7a and b.

The condition most frequently found is that shown in figure 9. The extreme cleavage polyembryony shown in figure 16 was found in about 10 per cent of the ovules of *P. urbanii*, and in a majority of the preparations there was some cleavage polyembryony, since some of the secondary embryos were found on the isolated ends of prosuspensor cells.

Figures 9, 10, 13, 15, and 17 show various stages for the terminal embryos in which massive secondary suspensors have appeared. A few embryos larger than these were obtained, but none were in the stage showing the organization of the meristems which give rise to the root tip, stem tip, and cotyledons. Figures 11 and 12 show the upper parts of prosuspensors with embryos formed in the position of rosette cells. Their origin is obscure. Sometimes the nuclei of prosuspensor cells, especially those that stay short, may remain in this upper region. Figures 11 and 12 both show this condition, also some of the upper ends of the prosuspensors in figure 9. There is a possibility that embryos may arise from the upper ends of



FIGS. 13-16.—*Podocarpus urbanii*: Figs. 13, 14, many-celled embryos on tips of prosuspenders, derived from 2 and 3 binucleate units; $\times 120$. Fig. 15, later stage showing massive secondary suspensor of embryonal tubes entangled with ends of prosuspensor cells (*ps*); $\times 70$. Fig. 16, 2 embryo systems under conditions of general cleavage polyembryony, with many embryos on ends of isolated prosuspensor cells; $\times 70$. Fig. 16a, prosuspensor cells belonging to fig. 16, detached during dissection; larger embryo probably one of several derived from terminal binucleate cells; $\times 70$. Fig. 16b, enlarged view of one terminal embryo showing apical cell in optical section; $\times 140$.

some of these, and the embryos of figures 11 and 12 seem to conform to such an origin. Occasional rosette cells may have failed to elongate, but none were found in several embryos corresponding in stage to that of figure 7.

PODOCARPUS MACROPHYLLUS MAKI.—The species labeled *P. chinensis* in the conservatory of the New York Botanical Garden fits PILGER's description (19) of the subspecies *maki* (Seib.) very closely. It is rather certain that the plants in Darlington, North Carolina, which furnished the embryological material for COKER's investigation belong here. As PILGER considers *P. chinensis* a synonym under *P. macrophyllus maki*, the latter name is used here.

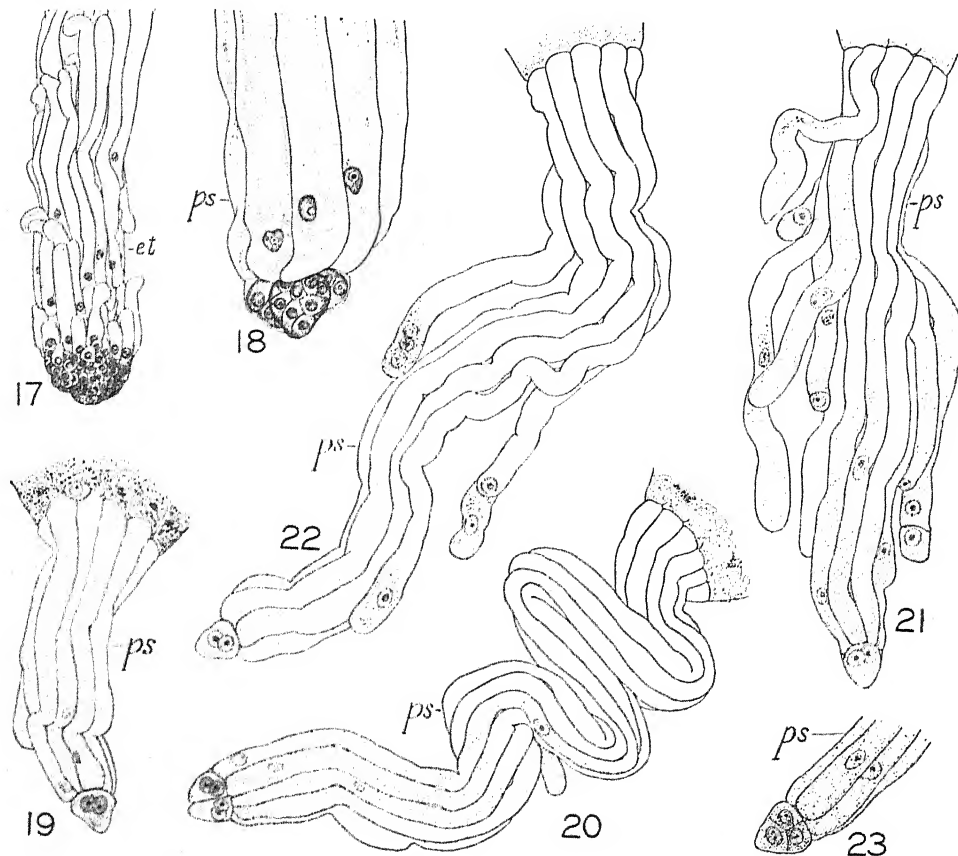
A few stages in the proembryo of this species were described by COKER (8), who reported sixteen free nuclei before cell walls appear. He shows two figures with the prosuspensor beginning to elongate, both of which would precede figure 19.

Recently TAHARA (26) described the proembryo of *P. macrophyllus* from material in Japan. He states that the proembryo forms walls after sixteen free nuclei have been formed, and his account agrees in its essentials with that of COKER (8) for this species. TAHARA shows definitely that in another species, *P. nagi*, the binucleate cells are so organized from the mitosis of a uninucleate walled cell of the proembryo. He shows also in his textfigure 4 that two binucleate cells may be formed occasionally in the proembryo of *P. macrophyllus*. Thus there is no occasion for assuming that a single binucleate cell ever splits longitudinally to form two units, where these are observed in later stages.

Figure 19 shows a deposit forming above the prosuspensor, faintly coral-like, in a stage of formation of the callose plug described by COKER (8). This deposit is also shown in figure 20, but is not shown in most of my other preparations because these plugs had been removed during dissection in a search for rosette cells. Figure 19 shows a single isolated rosette cell, and a few similar rosette cells were found in other embryo systems, but a tier of rosette cells is absent. COKER (8) speaks of a "rosette," but refers to the free nuclei situated above the prosuspensor, which disintegrate when the callose plug is formed. Most investigators on conifer embryogeny have used the term rosette for a tier of walled cells in the original sense of MIRBEL and SPACH (18), but of course COKER's rosette serves to designate its position, if one were present.

Figure 20 shows two binucleate cells at the tip. These may have had this organization in the proembryo. Usually only a single binucleate embryonic cell is found (figs. 19, 21, 22), and when this cell passes into the next stage it forms a 4-celled embryo with the cells arranged in tetrad form (fig. 23). The mitotic figures of this division were not actually observed in *P. macrophyllus maki* but were in *P. totarra*. A number of the 4-celled stages similar to that of figure 23 could be found, but it happens that none were observed in later stages with double cell masses that might have come from the two terminal cells shown in figure 20.

Figure 21 shows eight of the fourteen cells of the prosuspensor separated from the terminal binucleate cell, so that only six are left in contact with it. Two of these cells have already formed at their ends embryos which resemble ones with primary suspensors. In figure 22 one of the prosuspensor cells has three nuclei without walls between, and another has an elongated cell in place of a short em-



FIGS. 17-23.—Figs. 17, 18, *Podocarpus urbanii*: Fig. 17, terminal embryo slightly 2-lobed on massive secondary suspensor; $\times 70$. Fig. 18, terminal group of 3 embryos, each with about 16 cells; $\times 140$. Figs. 19-23, *P. macrophyllus maki*: Fig. 19, earliest stage with single rosette cell, prosuspensor bearing single terminal cell. Fig. 20, prosuspensor bearing 2 terminal binucleate cells; $\times 140$. Figs. 21, 22, detached prosuspensor cells forming embryos. Fig. 23, embryonic tip now 4-celled; $\times 140$.

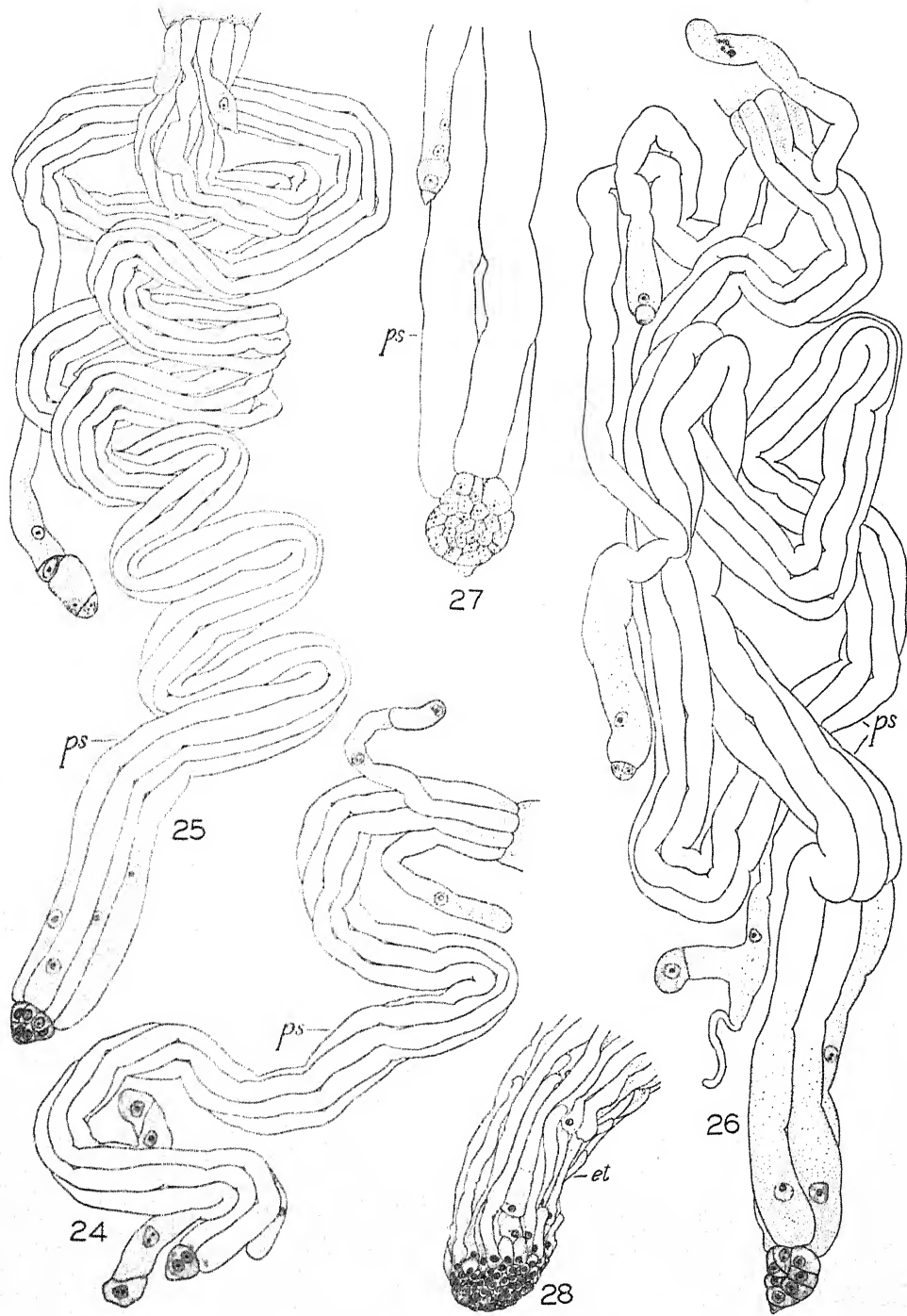
bryonic cell, indicating possible variability in the manner of forming the embryos on the ends of prosuspensor cells. Usually such an embryo does not become binucleate, and—as in the case of *P. urbanii*—the resulting embryos often become abnormal in appearance. Figures 25-27 not only show variations in these secondary embryos but also indicate that they may not be expected to be successful in competition with the larger embryo situated on the end of the prosuspensor.

Figure 25 shows a separated prosuspensor tube which bears two secondary embryos on its end and shows also some very short prosuspensor cells in the rosette region. The small embryo borne on the prosuspensor with an "appendix" in figure 26 is especially interesting. This prosuspensor cell did not elongate fully and the peripheral tubes completely surrounded it for a time while it developed a tapering end. In the twisting of the prosuspensor and the breaking away of peripheral tubes, this cell developed a tube leading to the surface, on the exposed end of which an embryo was formed. Later this tube became liberated, and may have been further loosened during dissection to give the appearance shown in this figure. TAHARA (26) observed a similar breaking away of cells of the prosuspensor on the ends of which embryos had been formed.

Several stages in the terminal embryo are shown in figures 23, 25, 26, 27. The persistence in these figures of the pointed end found in the earlier binucleate stage gives assurance that a single terminal embryo initial usually does not split into several smaller units.

While the stage shown in figure 27 is still far from differentiating the meristems of its members, there is no doubt that this mass of cells will contribute to the formation of a single embryo. The more mature stages of *P. macrophyllus* are shown by STILES (23). In the cases with single binucleate cells, simple polyembryony is the rule only so far as the terminal embryo is concerned. However, the occurrence of two binucleate cells (fig. 20) would provide exceptions to this rule, and the potentiality of embryo formation in other cells is demonstrated by any cell of the prosuspensor which becomes isolated from the others. The occurrence of these secondary embryos therefore results in a general condition of determinate cleavage polyembryony for *P. macrophyllus maki*.

PODOCARPUS TOTARRA.—This species illustrates another example of the Eupodocarpus type of embryogeny. The proembryo of this species, including *P. nivalis*, was described by SINNOTT (22) as having sixteen free nuclei, which become arranged in three tiers with walls forming before another division takes place. The terminal embryonic unit is usually a single binucleate cell, above which are 7-9 prosuspensor cells instead of 14; and the remaining nuclei, which must therefore be less than the number of prosuspensor cells, form a tier of relict nuclei above the prosuspensor tier and soon disintegrate. As in the preceding species, there is no tier of walled rosette cells, neither were isolated rosette cells observed. It appears from SINNOTT's description of the proembryo, supported by the fact that 7-10, usually 8, prosuspensor cells are observed, that the relict nuclei situated above the prosuspensor must be few, unless the number of free nuclei formed is not strictly sixteen. In any event the proembryo of *P. totarra* seems to differ somewhat from that of *P. macrophyllus maki* (8), a difference which is reflected in the smaller number of cells observable.



FIGS. 24-28.—Figs. 24-27, *Podocarpus macrophyllus maki*: Successive stages, with terminal embryo 8-celled in fig. 25; 16-celled in fig. 26; about 60-celled in fig. 27; $\times 140$. Fig. 28, multicellular terminal embryo of *P. urbanii*; $\times 70$.

Figure 29 shows the entire section of an ovule of *P. totarra* containing an embryo which is in the stage of figure 31. With the exception of the embryo, the drawing was made from a section of the ovule. The embryo was drawn from a dissected preparation of the same stage in order to show more effectively the coiled prosuspensor. The ovule gives the appearance of having a double integument, but the outer fleshy layer is the epimatium or equivalent of the ovuliferous scale, which—as GIBBS (12) and SINNOTT (22) have shown—is fused with and completely surrounds the ovule. Figure 29 corresponds closely to SINNOTT's photomicrograph (pl. VI, fig. 7). SINNOTT (22) has given the comparative details in the vascular anatomy of the ovules of the Podocarpaceae for a considerable number of genera and species.

Figure 30 is a stage in *P. totarra* which follows the oldest stage of an early embryo illustrated by SINNOTT (22, pl. VIII, fig. 32). The latter shows a few relict nuclei above the prosuspensor, but in figure 30 such nuclei could no longer be found. A number of the prosuspensor cells have already broken away. Figures 31, 35, and 37 show successive stages in the coiling of the prosuspensor. In figures 35 and 37, two embryo systems are included. Although the prosuspensor tubes break away as they do in the preceding species, little was found to indicate that they actually form secondary embryos. An earlier stage than figure 30 was dissected from an ovule of *P. hallii* (fig. 40). This embryo also has a single binucleate cell at the tip and is practically the same as *P. totarra*.

Occasionally two terminal binucleate cells were observed, even in stages similar to those shown in figures 30 and 31. Figure 34 shows the terminal embryo when it has become 4-celled. Figure 32 shows the end of an embryo system which had two terminal binucleate cells, one of which remains in this condition and the other has divided to form four cells. In this instance the four cells are not in tetrad form, so that only two of them could be shown in the drawing. Figure 33 is also a system with a double tip. The embryo in the background, of which only the edge is shown, is 4-celled, while the one in the foreground shows each nucleus of the binucleate cell in mitosis. The lower mitotic spindle is in telophase and shows the line of a new cell wall which is forming on the spindle between these two nuclei. The other spindle is in late anaphase with the axis of its spindle foreshortened. Both spindles have an abundance of spindle fibers passing between the newly formed nuclei, but the phragmoplastic spindle fibers that persisted from the previous mitosis (shown in some of the binucleate cells, such as in figure 6a) are still to be seen between these two newer spindles.

No doubt the wall between two binucleate cells is delayed until after this pair of spindles has given rise to four nuclei. The 4-celled stage in the terminal embryo is therefore derived directly from the single binucleate cell. It is probable that in all podocarps the binucleate cells directly form similar 4-celled groups. Figures 34

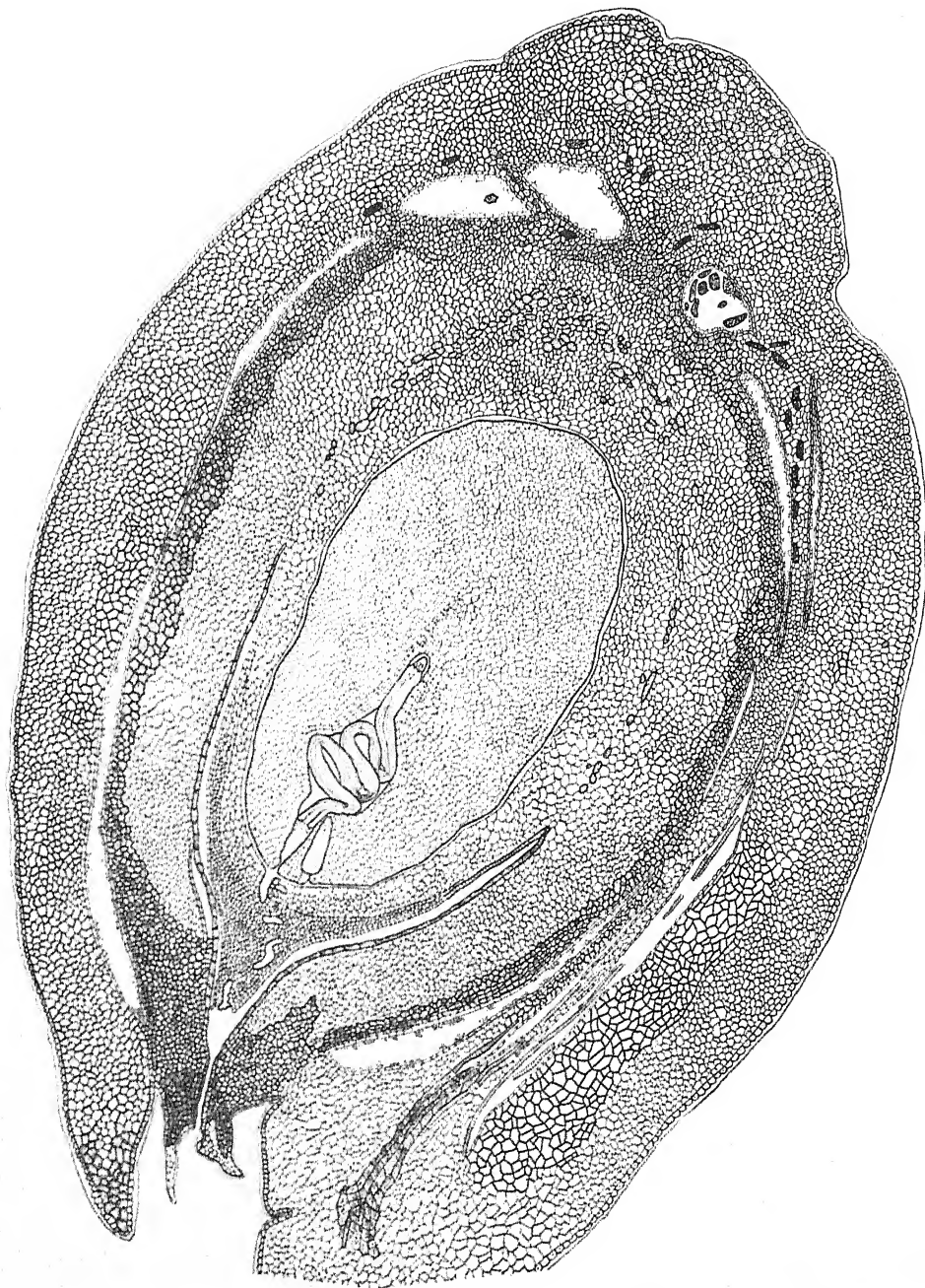
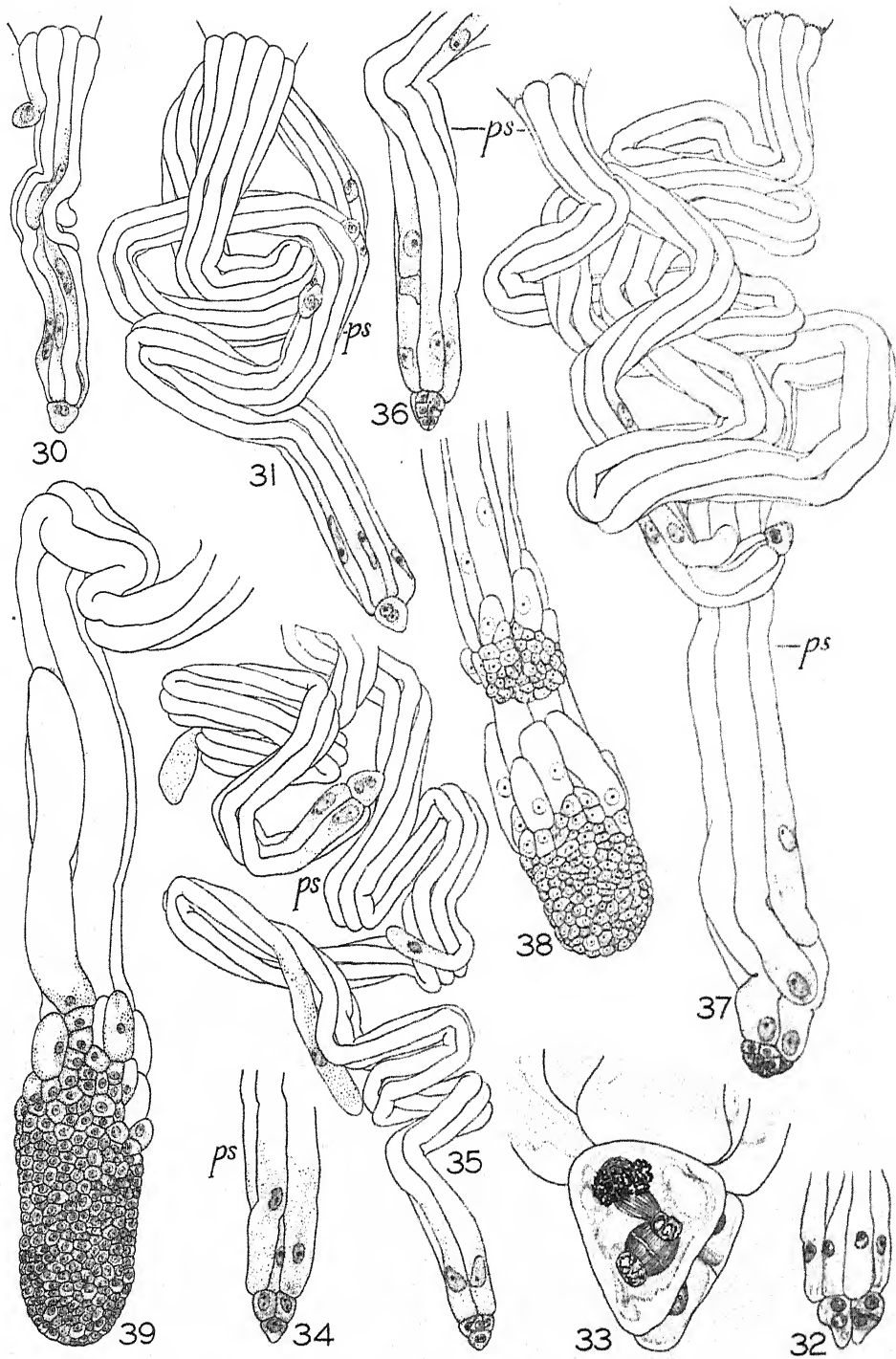


FIG. 29.—Section of entire ovule of *Podocarpus totarra*. Embryo system as found in dissected preparation drawn in place of incomplete embryo system. So-called ovuliferous scale is outer integument which envelops ovule; $\times 33$.



FIGS. 30-39.—*Podocarpus totarra*: Fig. 30, early stage with single binucleate cell borne on prosuspensor; also several cells detached from prosuspensor; $\times 120$. Fig. 31, later stage after great elongation of prosuspensor; $\times 120$. Fig. 32, tip of prosuspensor bearing 2 embryonic units, 1 still binucleate, other 4-celled, only 2 cells showing; $\times 140$. Fig. 33, highly magnified double tip showing 2 division spindles as both nuclei of binucleate cell form 4-celled embryo. Note older phragmoplastic fibers extending between both spindles. Edge of 4-celled embryo shown below; $\times 650$. Fig. 34, 4-celled embryo derived from binucleate cell; $\times 120$. Fig. 35, same; $\times 120$. Figs. 36, 37, 8-celled and 12- to 16-celled embryos; $\times 120$. Figs. 38, 39, later stages forming massive secondary suspensors; $\times 120$.

and 35 show 4-celled terminal embryos; figure 36 has an 8-celled embryo; and figure 37 a 12- to 16-celled embryo. In these early stages of cell formation the total size of the embryo does not increase greatly until many cells have been formed.

The stages between figure 37 and the embryos of figures 38 and 39 were not available for study. Figure 38 shows the terminal embryo separated from a smaller cell mass by its secondary suspensor (*et*). This condition was observed several times. Figure 39 is one of the oldest embryos dissected and shows in optical section that the organization of the plerome of the root tip is just beginning. Except for the cases in which two binucleate cells are found on the end of the prosuspensor, the embryogeny of *P. totarra* usually follows a program of simple polyembryony. The proembryo seems to have undergone some kind of reduction from the type of development shown in other species of *Podocarpus*, and the later stages appear to have become modified so that cleavage polyembryony has become more or less suppressed.

PODOCARPUS GLOMERATUS.—The embryo of this South American species was examined from herbarium material only. The writer examined an undated specimen collected by A. Rimbach (626) "in the valley of the Chimbo river near Guaranda, alt. 2800 m. on the slopes of the western Cordillera" of Ecuador (Field Museum). The embryo complex dissected from this specimen included two systems, the smaller of which was incomplete. The larger prosuspensor in this complex had about twelve cells in its upper part, where it joined the base of the archegonium, and of these about nine extended throughout the entire length to the embryonic tip. The latter was composed of three separate and distinct but closely grouped embryos, which if drawn would give a figure similar to figure 18. The three embryos had all advanced to the 8-celled stage, but they retained the original contours of the three binucleate cells from which each was derived, so that it was evident that three existed in an earlier stage (such as that shown in figure 7*b*). The embryogeny of *P. glomeratus* may be presumed to be very similar to that of *P. urbanii*.

PODOCARPUS CORIACEUS.—The only stages observed with early developing embryos came from a herbarium specimen collected by H. Pittier (8298) in Venezuela, December 26, 1929—"Altos de Galipan, Cerros del Avila, alt. 1600 m." Three embryo systems on relatively short and untwisted prosuspensors were found in the only ovule examined. Their respective embryonic tips were composed of three, three, and two embryonic units, some of which appeared to be past the binucleate stage. The prosuspensors at the top or archegonial ends had about 11-12 cells. Provisionally one would describe the general embryogeny as resembling that of *P. urbanii* more closely than that of any other type which has been described.

PODOCARPUS PURDEANUS.—Two ovules were examined from herbarium material collected by Britton and Hollick (2798) in Jamaica, April 6-7, 1908. One ovule

contained a complex of two embryo systems. The two prosuspensors had 11-12 cells at the archegonial end. The number of binucleate cells or units borne on the end of the prosuspensors could not be determined. The cells of the prosuspensor had become separated, showing a condition similar to that in figure 16, with the terminal embryo larger than that shown in figure 17. The partially disintegrated remains of thirty-three or more small embryos could be observed among the embryo complex of prosuspensor cells.

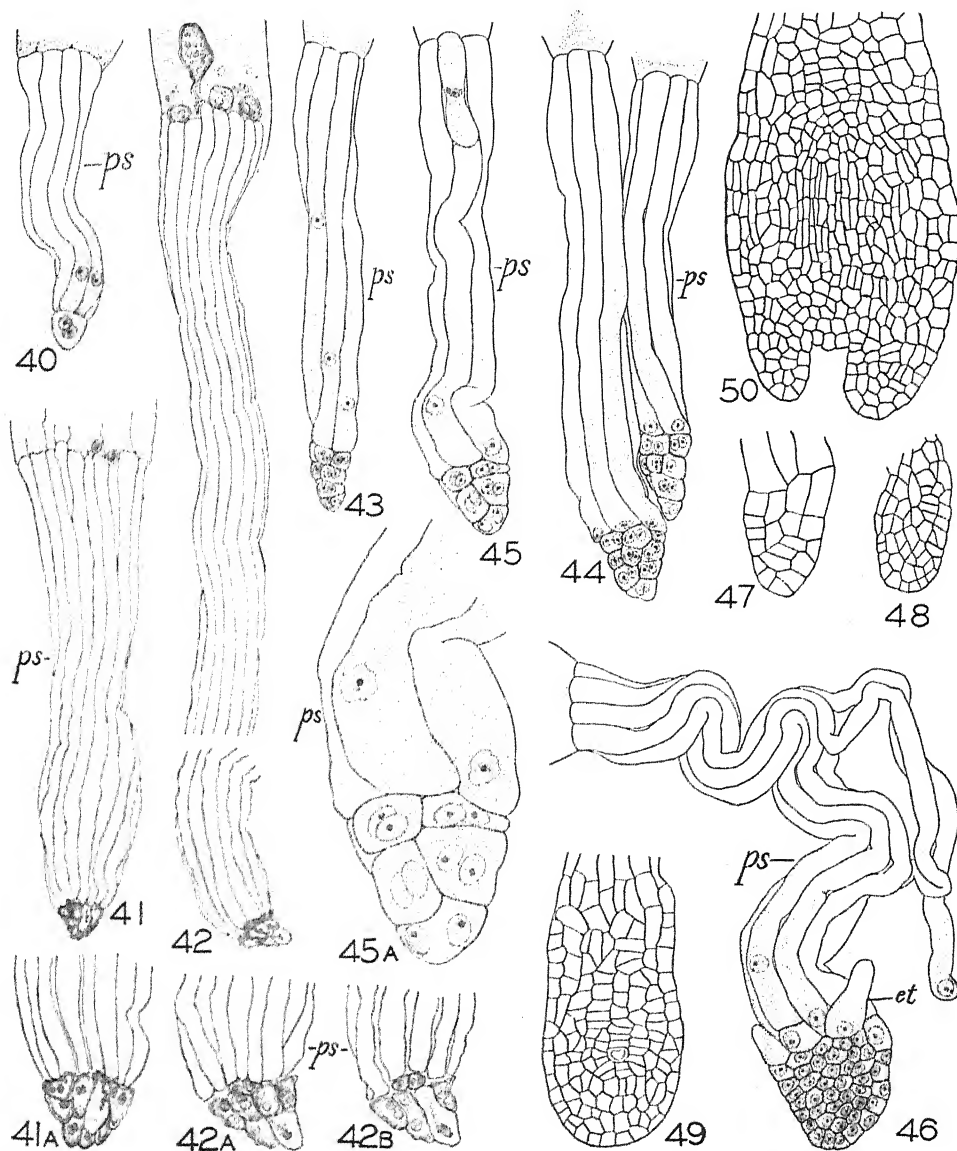
The other ovule had only a single embryo system, with the terminal embryo at about the same stage or slightly larger. The remains of about fourteen small embryos could be counted in and around the tangle of separated strands of the prosuspensor.

PODOCARPUS MATUDAI.—Only a single ovule of this Mexican species was examined. This came from the Matuda collection (956), Mt. Avado, somewhere in Chiapas, the extreme southern state of Mexico. The embryo complex contained four embryo systems, each with about twelve prosuspensor cells shown at the top. The lower end of the prosuspensor of the largest system showed nine cells extending to the tip, where the largest embryo had been broken off. However, this largest terminal embryo was lost in dissection. One of the smaller aborted embryo systems had a double tip. In one of these two cells the binucleate condition could be observed distinctly. Another one of the aborted system had an embryonic tip which showed several cells, but the nuclei could not be seen.

All the Central and South American species of *Podocarpus* thus far examined (including several species not specially cited here) had about twelve prosuspensor cells at their archegonial ends. None of them agree with the condition found in the 8-celled prosuspensors of the New Zealand species, *P. totarra*, *P. hallii*, and *P. nivalis*.

PODOCARPUS, SUBGENUS STACHYCARPUS

PODOCARPUS GRACILIOR.—This species is placed in the *Stachycarpus* subgenus by PILGER (19, 20). The specimen, which contained a pair of early embryos of great diagnostic value, came from collections of Edgar A. Mearns, expedition to South Africa (382), collected on June 7-8, 1909, "between Aljoro-o-Nyou and Narok river at an altitude 2000 m." One of the embryo systems is illustrated in figure 41, with an enlarged detail of the tip shown in figure 41a. There are about twenty cells in the prosuspensor, which bears ten cells at its tip, and in most of these cells the two nuclei could be recognized under oil immersion. This remarkable embryonic type was observed and reported in a slightly earlier stage for *P. usambarensis* (4). The embryo of figure 41 has a cell in which the protoplasm has collapsed and the cell is nearly empty. There is a cell in the interior completely surrounded by the others. No later stages of *P. gracilior* were obtained, but four embryos of *P. amarus* belonging to this taxonomic category were obtained from



FIGS. 40-50.—Fig. 40, early embryo of *Podocarpus hallii*; $\times 120$. Fig. 41, same of *P. gracillior* with 18-22 prosuspensor cells, bearing about 10 binucleate cells below; $\times 80$. Fig. 41a, enlarged view of lower end of fig. 41; $\times 155$. Fig. 42, early embryo of *P. nankoensis*. Prosuspensor broken in dissection, but nearly all is represented. It is made up of 18-20 cells, with a few isolated rosette cells above; it has also a club-shaped ball of cytoplasm showing 11 nuclei that may have come from relict nuclei of egg cavity above prosuspensor, shrunk thus during drying; $\times 80$. Figs. 41, 42, from dried herbarium material. Fig. 42a, b, two views, tip of fig. 42, in different planes of focus; $\times 155$. Figs. 43-50, *Phyllocladus alpinus*: Fig. 43, earliest stage, 5 prosuspensor cells, bearing 10 binucleate cells below; $\times 110$. Fig. 44, 6 prosuspensor cells at left and 5 at right (ps), each bearing 11 binucleate cells; $\times 110$. Fig. 45, 5 prosuspensors bearing 9 binucleate cells; $\times 110$. Fig. 45a, tip of fig. 45 showing cytoplasmic strands between nuclei of terminal cell; $\times 250$. Fig. 46, oldest stage dissected. Figs. 47-50, successive stages from sections.

the A.D.E. Elmer collection of plants of the Philippine Islands (11862). This specimen was collected in September, 1909, on the island of Mindanao, Todaya (Mt. Apo), District of Davao.

PODOCARPUS AMARUS.—Three ovules were dissected. One endosperm contained a complex of two embryo systems. The prosuspensor of each system showed that it was composed of about twenty elongated cells at the upper or archegonial end, and it had elongated to a length of 7-8 mm. On the lower end of one of these systems, three embryos were attached by their secondary suspensors. The terminal and largest embryo had a secondary suspensor nearly 2 mm. long and was a conical mass of embryonic tissue of seventy or more cells. The next embryo, well separated but closely behind the first, was only slightly smaller, and the third was composed of about fifty cells. The secondary suspensors of all three were closely interwoven distally, and near their junction to the prosuspensor 7-8 embryos were found which were much smaller and had not developed secondary suspensors. Nearly all these 10-11 embryos were situated so that they seemed to belong to the same system.

There were six separate embryos grouped near the end of the other prosuspensor. The largest of these had about twenty-five cells and was just beginning to form its secondary suspensor. Very few nuclei could be seen in cells of the prosuspensor, but the entire complex was covered with many starch-filled endosperm cells. It appeared that a few small rosette embryos were present, but this region was covered with a deeply stained basal plate deposit which obscured these details.

Both of the other two ovules of *P. amarus* whose endosperm was dissected contained single embryo systems; both showed about twenty cells in the upper ends of their prosuspensors, and in one of them there was a small rosette embryo, probably derived from an isolated rosette cell. One of these systems had elongated to about 8 mm. and was tipped by a group of six separate embryos, the largest of which was beginning to form its secondary suspensor and was composed of about thirty-five cells. The other embryos of this group were smaller, and a few of them were somewhat irregular in shape. About 1 mm. back from the tip was the largest embryo, composed of about forty-five cells but without a secondary suspensor. There were a few other very small embryos, some of which appeared to have been formed on the ends of some of the prosuspensor cells.

The other system was 9 mm. long but bore four terminal embryos on secondary suspensors. These embryos had slightly unequal, entangled secondary suspensors; the largest had about eighty cells, the others were estimated at sixty-five, fifty, and forty cells each. About 0.5 mm. back of this group was an embryo of about twelve cells and several smaller embryos of only a few cells, some of which appeared to be so situated that they may have been derived from the ends of prosuspensor cells. Some of the prosuspensor cells had formed internal chains of embryonic cells.

If one may assume that the embryos of *P. usambarensis*, *P. gracilior*, and *P. amarus* are all essentially similar and present a general outline of the type of embryogeny in this group, the conclusion is that these have cleavage polyembryony. Each of the binucleate cells at the end of a prosuspensor cell in *P. usambarensis* and *P. gracilior* gives rise to a separate embryo, unless it becomes aborted in an early stage of development. The numerous larger embryos in *P. amarus* must have been derived from this number of binucleate cells.

PROTOPODOCARPUS, SECTION NAGEIA

The embryogeny of *Podocarpus nagi* has been investigated recently by TAHARA (26), who described the proembryo as having thirty-two free nuclei before walls are formed. Seven to nine of these nuclei become surrounded by walls, forming a tier of cells in the lower end of the archegonium. Above this tier the remaining nuclei become organized into another tier, with walls between the nuclei but remaining open to the egg cavity above. The next division of the nuclei forms a tier of 23-25 prosuspensor cells with an open tier of relict nuclei above. No rosette is formed and no isolated rosette cells were observed. The nuclei of the lower tier divide to form embryo initials which remain binucleate for the early period of elongation of the prosuspensor. In this account TAHARA has answered the question of the precise origin of the two nuclei of the binucleate cells. His series of figures is complete enough to demonstrate that the two nuclei in a binucleate cell are not some of the thirty-two free nuclei held over but all originate from a cell with a single nucleus. This provides the basis for assuming that all binucleate cells in the Podocarpaceae have a similar origin.

The embryogeny of the Nageia group is very similar to that of *P. usambarensis*, *P. gracilior*, and *P. amarus*. An early embryo of *P. nankoensis* is shown in figure 42. Here the prosuspensor, bearing a group of embryonic cells, is in an early stage of development. This embryo system, broken during dissection, has only a negligible portion of the prosuspensor missing. There are 19-20 prosuspensor cells in its upper part, with several rosette cells; at the embryonic end at least eighteen could be counted. The embryonic tip is composed of a group of 10-11 binucleate cells shown in surface view in figure 42a, and in a median plane of focus in figure 42b, where only a few cells remain hidden. One of the largest cells, in a central position, has become abortive and lost nearly all of its protoplasmic content. Two very small cells are situated in the interior, completely surrounded by prosuspensor cells above and by neighboring binucleate cells below. Owing to shrinkage, they show through in the surface view; in living condition they would be covered on all sides. It is only with respect to these small cells that this embryo differs from that of *P. gracilior* (fig. 41) and of *P. usambarensis*.

Three embryo systems were observed in this stage. One of the embryos of *P.*

nankoensis, not illustrated, had no internal binucleate cell and another appeared to have only one such cell, which was completely surrounded. Both of these unillustrated embryos were without the abortive cell shown in figures 41 and 42.

These variations may indicate that the exact arrangement of the cells and the stage of abortion of some of them are variable and may be relatively unimportant. The rosette region at the top of the prosuspensor in figure 42 appears to have four rosette embryos. One of them extends upward and has about twelve nuclei in a dense mass of cytoplasm, surrounded by a common wall but with no walls separating them. This may not be a true embryo but an appearance due to some kind of artifact formed during drying of the specimen, in which the free nuclei in the egg cavity above the prosuspensor were collected into a ball of cytoplasm. One rosette embryo is 2-celled, two others remain single-celled. Rosette cells similar to the latter were observed in the embryo of *P. usambarensis* (4). There were rosette cells and embryos similar to the smaller ones in figure 42 on one of the other two embryo systems not illustrated.

While the embryos of *P. nankoensis*, *P. nagi*, and *P. blumei* which were dissected from herbarium specimens in later stages of development could be described and would provide all the evidence needed to conclude that cleavage polyembryony is found regularly in the *Nageia* group, it is deemed unnecessary to retain this portion of my description. The recent publication by TAHARA has shown this clearly from material properly killed and fixed. TAHARA's textfigure 12 shows the general condition, after the embryos derived from the binucleate cells have become separated on their own secondary suspensor. My material confirms his observations in all essentials; there is no doubt that cleavage polyembryony is the normal condition in the *Nageia* group.

The only difference in the observations of the writer made upon herbarium material indicates a smaller number of cells in the prosuspensor and a few more embryo initial cells in the terminal group. Occasionally one or two of the cells in the terminal group may be situated internally and completely surrounded by others, as shown in figure 42, 42a. TAHARA's figures, however, indicate that there may be more than nine; his textfigure 10a, showing parts of five cells in the lower tier in the same longitudinal section, may have more than nine, and his textfigure 10b shows a cell centrally placed so that it may be completely surrounded by others, as shown in figure 42, 42a.

The later embryo systems of the *Nageia* group, in which the surviving terminal embryos had become large and developed two cotyledons, usually failed to show more than a few of the remaining abortive embryos. With very careful dissection, it was usually possible to count the upper ends of the prosuspenders so that the number of embryo systems could be determined.

In one ovule five embryo systems were found with the remains of more than fifty

small aborted embryos; an ovule with six embryo systems had more than forty aborted embryos; another with four systems had more than twelve. *P. blumei* appeared to have similar conditions. In all the species of the *Nageia* group there were two cotyledons, but one out of three of the embryos of *P. blumei* had three.

EMBRYOGENY OF PHYLLOCLADUS

The first account of the special morphology of *Phyllocladus alpinus* was given by KILDAHL (14). This was incomplete but was amplified considerably by YOUNG (27), who confined her account to the stages through fertilization. KILDAHL had included a few stages in the proembryo up to the stage with eight free nuclei, with no indication that wall formation was imminent at this stage. It is likely, therefore, that sixteen free nuclei are followed by wall formation. Since the earliest embryos available had 5-6 cells in the prosuspensors, bearing 9-11 binucleate embryonic cells, the events are probably as follows: sixteen free nuclei are organized with walls arranged more or less in tiers of 5, 4, 3, 3, 1 or 6, 4, 3, 2, 1 cells, with the upper 5- or 6-celled tier still open above. This is followed by a nuclear division in each cell, the upper tier giving rise to 5-6 prosuspensor cells and an equal number of relict nuclei (which soon go to pieces) in a tier without walls. The 9-11 walled cells below the prosuspensor tier would thus each become binucleate, and—following elongation of the prosuspensor—an embryo similar to figure 43 would result. This is in general agreement with LOOBY and DOYLE's account of the proembryo of *Saxegothaea* (10, 17), which differs only in having a shorter prosuspensor made up of a smaller number of cells.

HOLLOWAY (13) in his account of the embryogeny of *Phyllocladus alpinus* seems to have overlooked the binucleate condition in the early embryonic cells. Figure 43 represents one of the earliest stages obtainable of the embryo of *P. alpinus*. This example has five cells in its prosuspensor and bears ten binucleate cells below in four tiers of 4, 3, 2, and 1 cells each. Figure 44 shows two adjacent embryo systems. The larger, at the left, has six prosuspensor cells and 10-11 binucleate embryonic cells in four tiers, and the one at the right with five cells in its prosuspensor has ten embryonic cells, nine of them binucleate and two of them definitely uninucleate. Figure 45 has five cells in its prosuspensors (or six counting the short one above) and 9-10 binucleate cells below them. The enlarged drawing of this tip (fig. 45a) shows the telophasic spindle fibers passing between the two nuclei, as in several of the cells in *Podocarpus dactyloides* (fig. 6a).

Figure 46 shows the oldest embryo obtained from dissected material. In this at least six cells are found in the prosuspensor. The embryo has become large and multicellular, and the beginning of elongation of the embryonal tubes to form the secondary suspensor is shown. Although *Phyllocladus* has a very short prosuspensor, this member appears to be relatively longer than that of *Saxegothaea* (10).

Several stages in the embryogeny of *Phyllocladus alpinus* were also studied from serial sections. Four of these are shown in figures 47-50. Figure 47 is a stage just after the embryonic cells have all become uninucleate, and would follow one of the embryos shown in the dissected stages such as figure 44 or 45. It is probable that each of the binucleate cells forms four uninucleate cells at a single step, as was shown in figure 33. This, accompanied by a slight enlargement, would result as shown in figure 47, in which all cells were uninucleate. Here the apical cell arrangement with an appearance of segments may be due to an origin of groups of four uninucleate cells from each of 9-11 binucleate cells arranged in a conical mass. In any event the mass shown in figure 47 did not come from a single apical initial cell, and an apical cell need not be present to give this appearance of segmentation.

The next stage (fig. 48) differs little from that of figure 47 in its total size. There are, however, more than twice as many cells. An apical initial cell was not clearly recognizable in this stage. Figure 49 is a median section in a later stage in which the cells are larger, as well as more numerous, and the organization of the pleromic apex of the root is indicated by the centrally placed cell shown with a nucleus. All parts of this figure (about two-thirds of it) situated above this cell belong to the calypetroperiblem (6), which merges into the secondary suspensor, while the embryo proper is derived from the cells below this pleromic apex. If a circular line were drawn to include the lower parts of this figure and the cell with the nucleus included on its circumference, all the cells which give rise to the essential parts of the later embryo except the calypetroperiblem would be included.

Figure 50 is a section through the later embryo after the cotyledons have appeared, but it is not a perfect median section, so that it shows only imperfectly the region of the pleromic apex of the root tip, which is now situated about three-fourths of the distance from the lower end of the figure. Two cotyledons are shown, and (on the left side) a series of narrow elongated cells mark the edge of the plerome in the hypocotyl region. The stem tip does not appear to be elevated at this stage. This is the largest embryo which was available for study. One stage shown by HOLLOWAY (13) has the same width and differs only in being somewhat longer throughout.

Discussion

Before proceeding with the discussion, it is desirable to correct some errors and misinterpretations which have appeared in certain accounts. I have also fallen into an error in my interpretation of the embryogeny of *P. macrophyllus maki*—given after COKER (8) under the name *P. coriacea*. The diagram published elsewhere (1, fig. 18) is incorrect. No primary suspensors are formed by the terminal embryos borne on the end of the prosuspensor. The small embryos that appeared on the ends of single suspensor elements were erroneously interpreted as borne on

primary suspensors. I know now that the primary suspensor is not usually present in any species of *Podocarpus*, but there are secondary embryos formed on the ends of detached cells of the prosuspensor, and these might resemble embryos borne on primary suspensors.

The problem of an apical cell in *Phyllocladus* is somewhat related to the binucleate cells. It may be assumed from the observations on *P. totarra* (fig. 33) that each binucleate cell gives rise to a group of 4-walled cells at one step. After a similar division, the embryo of *Phyllocladus* would have 40-44 cells grouped in 10-11 areas. These might appear as the segments resulting from apical cell growth. It is easily possible, therefore, especially where the shape of the entire embryonic mass of cells is conical, to find a terminal group of four tetrahedral cells that give the appearance of an apical cell which has produced a single-celled and 2-celled segment next to it, with the other 4-celled groups assuming the appearance of older segments. Although I illustrated such an embryo (1, fig. 20D), pointing out that only the apical cell comes from the lowest binucleate embryonic initial and cannot be responsible for growth of all parts of the embryo, I am convinced now that the existence of a true apical initial cell in *Phyllocladus* is very doubtful; if present at all, it cuts off only a small number of segments before it disappears.

In regard to HOLLOWAY's error (13) in failing to observe the binucleate cells in the early embryo of *Phyllocladus*, it should be stated in justice to him that it is very difficult to recognize this condition in serial sections alone; it might have been overlooked in the sections left from YOUNG's investigation (27), which I have before me.

The differences in embryogeny within the genus *Podocarpus* are remarkable. Extremes are found in the number of prosuspensor cells, from about 19-22 in *P. usambarensis* (4) to seven, eight, or nine in *P. totarra* and *P. hallii*. If the entire family is considered this variation is still greater, since the number of cells in the prosuspensor of *Phyllocladus* drops to five; and in *Saxegothaea*, according to DOYLE and LOOBY (10), some embryos have four and others only three. Likewise the variation in the length of the prosuspensor is extreme. The longest known are found in the plants closely related to *P. usambarensis*, and in the *Nageia* group, the ones having twenty or more cells in the prosuspensor. For example, PILGER (20) mentions that he found a suspensor system in *P. amarus* which was 30 mm. long. Of course this doubtless included a considerable portion of secondary suspensor, even though PILGER mentioned that the embryo at the end was still very immature. Within the genus *Podocarpus* the prosuspensor is usually relatively long, but in some other genera, such as *Phyllocladus* and *Saxegothaea* (10), the suspensor is very short. In one of these two genera it reaches its known limit for shortness. There is also considerable variability in the behavior of the cells of the prosuspensor. They may remain closely attached to the terminal cell or group of

cells as in *Podocarpus spicatus* (4) and in *Dacrydium* (3), or they may become separated so that all or nearly all become embryonic as in *P. urbanii*.

The presence of 1-12 or more binucleate cells at the tip of the prosuspensor is another remarkable feature. Thus far, all podocarps examined in the proper stage have had binucleate embryonic cells. This list now numbers nineteen species when *Saxegothaea* is included, and embodies four genera of the family. Unfortunately SAHNI (21) makes no mention of the embryonic cells in his detailed study of *Acmopyle*, but his figures show that it has a long, well-developed prosuspensor, and his work proves on other grounds that this genus belongs to the Podocarpaceae. Likewise LAWSON (16) has overlooked this condition in *Microcachrys*, if it occurs there. His investigation, however, like that of HOLLOWAY (13) on *Phyllocladus*, was confined to the study of serial sections.

In spite of the variations within the family in the relative length and number of cells of the prosuspensor and in the number and arrangement of the binucleate embryonic cells, there is an orderly transition from one extreme to the other, which is not in great disagreement with the arrangement of genera, subgenera, and species. Some transfers in the taxonomic groupings within the genus *Podocarpus* will be necessary, but these will be discussed later.

If the primary comparisons could be made between the proembryos, this no doubt would afford the most satisfactory basis for the larger groupings. However, the proembryos of only five types have actually been observed. These are *Saxegothaea* (10, 17), *Podocarpus spicatus* (22), *P. macrophyllus maki* (8, 26), *P. nagi* (26), and *P. totarra* (22). With these as a background, a careful study of the early embryos of the remaining species makes it possible to estimate the probable proembryos in most of the remaining forms examined. This was done for *Phyllocladus alpinus* in an earlier part of this paper. It can also be done for *Dacrydium*.

Dacrydium has 9-11 prosuspensor cells, which—if produced in the usual manner—have had an equal number of relict nuclei in a tier above the prosuspensor. If walls are formed in its proembryo after sixteen free nuclei have appeared, and 5-7 cells arranged in two unequal tiers below are to form this number of embryo initial cells, this would leave 9-11 nuclei in a third tier, whose division would result in a prosuspensor tier and a tier of relict nuclei, with the 5-7 embryonic cells becoming binucleate. Variations are to be expected. An occasional nucleus in the upper tier which fails to divide and joins the group of relict nuclei would decrease the number of prosuspensor cells, etc. In the proembryo of *P. dacrydioides* a similar program would be expected, except that here the embryonic cells are fewer and would be arranged in a single tier. *P. usambarensis* and *P. gracilior* have early embryos which are very similar to *P. nagi* as described by TAHARA. All these must have a proembryo in which thirty-two free nuclei are formed before walls appear.

Of course, some of the proembryos of the Podocarpaceae just described are only

hypothetical. Several of those actually observed should be more fully investigated. Comparisons of the proembryos of this family cannot be made on the basis of fully known facts, but comparisons of the early embryos—in which the nearly comparable stages have actually been observed—are possible. Figures 51 and 52 show a series of diagrams, drawn to the same scale of magnification, embracing the types of early embryos found in the Podocarpaceae, so far as these have been investigated. The numbers of binucleate cells in the terminal group are shown by numerals placed below them and the numbers of cells in the prosuspensor by numerals above.

This series of embryos probably includes the extremes of embryonic types to be found in this family. The number of binucleate cells ranges from twelve or more in four tiers (fig. 51) below the prosuspensor, through about 7-11 binucleate cells all arranged in a single tier, down to the single binucleate cell shown at the right (fig. 52). One could not expect to find fewer than one binucleate cell at the tip, or to find fewer than three in the prosuspensor at the other extreme. The prosuspensor varies, not only in the number of cells of which it is composed but also in the extent to which these elongate. In future researches on podocarps, it may be expected that the remaining species will fit somewhere in this series. The order from left to right is in general agreement with the taxonomic arrangement of the genera and species within the family, but it includes two rearrangements in the groupings. *Dacrydium* is inserted near the middle of the series; and the *Nageia* group, which is placed beside it, must include also *P. usambarensis*, *P. gracilior*, and their close relative, which would be transferred from the subgenus *Stachycarpus*. It is evident that the genus *Podocarpus* breaks up into several smaller groups in a new arrangement, among which *Dacrydium* may be inserted.

The genus *Podocarpus* presents almost as great variety in embryogeny as the entire family Podocarpaceae. While figures 51 and 52 also include the three genera *Saxegothaea*, *Phyllocladus*, and *Dacrydium*, the addition of these does not greatly increase the range of types shown by the species of *Podocarpus*. This genus seems to embrace sufficient variety to deserve segregation into several genera, although such splitting would seem premature in the present state of our knowledge. It would also seem impractical, if the only differences are to be found in embryogeny. No doubt there are external taxonomic differences correlated with these variations in embryogeny. A careful study of the detailed morphology and embryogeny of all possible forms should be made; then the correlated external taxonomic differences will become apparent.

The question as to which embryogeny is the most primitive presents itself. The answer given from a study of proembryos would lead to the selection of a type close to *P. usambarensis* and the *Nageia* group, since this type would have thirty-two free nuclei before walls are formed, in closer agreement with the number found

in the Araucaraceae (7, 11) and *Sciadopitys* (25). The same selection is suggested when the series of early embryos is examined. The modification in evolution within the family seems to have taken place in both directions from near the center of the series shown by figures 51 and 52 combined. Whether the embryonic cells are arranged in a single tier or in two is relatively unimportant, since a progressive narrowing of the archegonia would bring about the change shown in the direction of *P. spicatus* and *Saxegothaea*. It is not likely that one would select either of the extremes in this series if the postulated prototype is to have features in common with other groups of conifers.

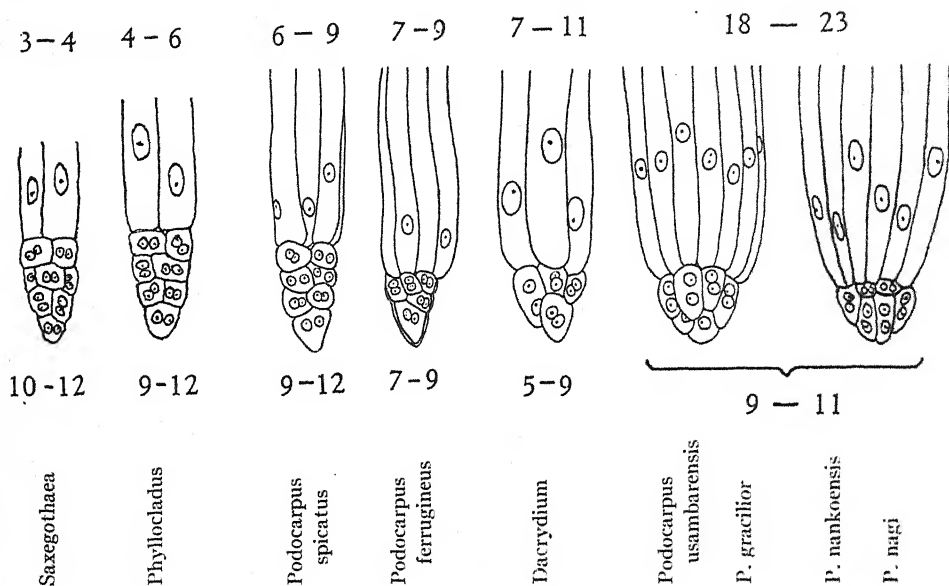


FIG. 51.—Diagrammatic comparison of early embryos of Podocarpaceae showing, by numerals placed above the figures, variations in number of prosuspensor cells and by numerals below the figures, variations in number of binucleate embryonic cells. The latter are arranged in 4, 3, 2 tiers or in a single tier (continued in fig. 52).

The embryogenies of *Araucaria* (7, 24) and *Agathis* (11) have been unique and puzzling. They could not be related closely to any other conifer, partly because the numbers of free nuclear divisions in the proembryo have greatly exceeded those known to occur in other genera and partly because the organization of the early embryo is unique. It is desirable, therefore, to digress in order to point out a feature wherein the embryogeny of some of the podocarps offers some clues in their similarities.

Figures 41 and 42 of *P. gracilior* and *P. nankoensis* show a plan of cellular organization very similar to the early embryo of *Araucaria* and *Agathis*. As

pointed out, these podocarps and also *P. usambarensis* (4) must have thirty-two free nuclei in their proembryos before walls appear. This is a condition nearer to the araucarians than is found in any other member of the Podocarpaceae. Furthermore, this cellular organization sometimes includes a few internal cells completely surrounded by others. In araucarians the number of cells included in this internal group is much greater than in these podocarps. They are all uninucleate; also the cells that form the cap are more numerous, and these are all uninucleate. The number of cells in the prosuspensor of the Araucariaceae and of these podocarps is about the same, and these are uninucleate in both families. *Araucaria* has simple

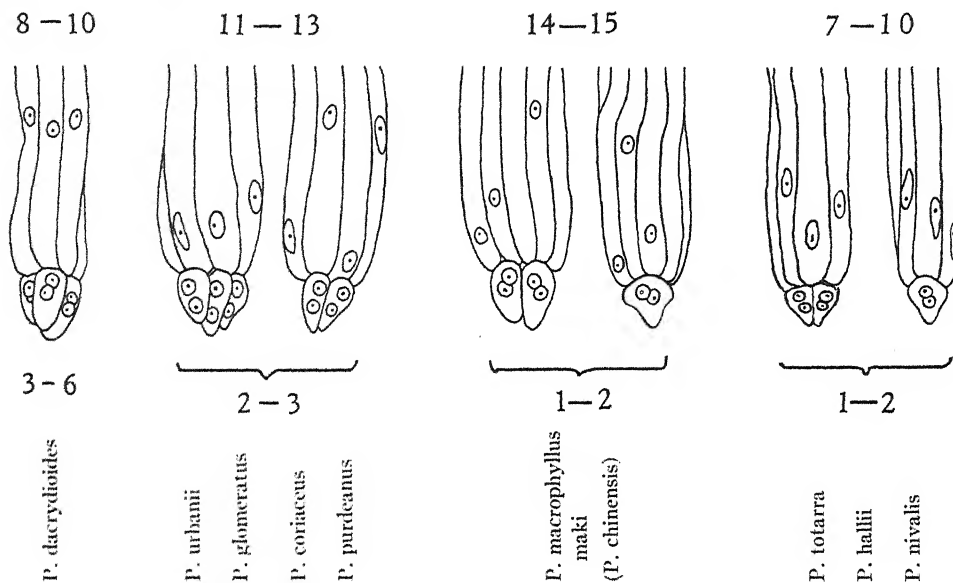


FIG. 52.—Comparison of early embryos of Podocarpaceae (continued). *Podocarpus* includes the widest range of variation found in any genus of Coniferales. In the above group the number of embryonic cells ranges from 6 to 1, all in a single tier. All diagrams are approximately to same scale.

polyembryony while the species of *Podocarpus* in question have cleavage polyembryony. Some of the binucleate cells in these podocarps may abort without forming embryos, while in *Araucaria* and *Agathis* all the peripheral cells that form the cap are abortive, and only the group of internal cells actually forms the embryo. While the embryogenies of araucarians and certain podocarps still stand far apart, these comparisons are the closest that can be made between the Araucariaceae and any other group of conifers. There are, of course, many other morphological similarities between these two families.

The genera and species which appear to have simple polyembryony are *Phyllocladus*, *Podocarpus spicatus*, *P. ferrugineus* (4), and *P. totarra*, together with the

forms closely related to the latter. Those with some degree of cleavage polyembryony are *Saxegothaea* (10), *Dacrydium* (3), *Podocarpus dacrydioides*, *P. imbricatus*, *P. urbanii*, *P. macrophyllus maki*, *P. glomeratus*, and *P. purdieanus*. In a number of these, simple polyembryony may be the apparent condition, for when cleavage polyembryony occurs it may be of the determinate type shown by *P. urbanii* and *Dacrydium* (3). The more open or indeterminate type of cleavage polyembryony occurs in the *Nageia* group of *Podocarpus*, including with them *P. usambarensis*, *P. gracilior*, and *P. amarus*. All of these forms thus far studied have cleavage polyembryony.

All the Podocarpaceae show a polyembryonic organization by the occurrence of the binucleate cells; those with more than a single binucleate cell are essentially polyembryonic and of the cleavage type. The podocarps appear to be polyembryonically compound. Whether an actual separation of the respective cell progenies of the binucleate embryonic units occurs where several are present makes no essential difference, except in the appearance of the embryonic complex. There may be a closed system in which the cleavage polyembryony does not always show its fundamental nature by separations of many small embryos. For example, the embryo of *Phyllocladus*, which produces only one embryo per fertilized egg, develops in such a manner that the progeny of only one of the terminal binucleate cells is likely to contribute the meristematic parts of the later embryo. The other binucleate cells contribute only to the secondary suspensor and calyptrorperiblem. In a strict sense, therefore, a form of determinate cleavage polyembryony is present even in the organization of this embryo, where competition between the parts has been pushed back to a very early stage and is so coordinated that it never comes to expression. Thus the embryogeny of *Phyllocladus* may be described as having simple polyembryony in spite of this polyembryonic organization. The same would be true of *Saxegothaea* in its normal development, in which, however, DOYLE and LOOBY (10, 17) found that some kind of cleavage polyembryony becomes recognizable in about 15 per cent of the embryo systems. As already pointed out, the embryogeny of *P. urbanii* shows cleavage polyembryony for the two or three embryonic binucleate cells. It has also embryos formed on prosuspensor cells as extensively as shown in figure 16 in about 10 per cent of the embryo systems, although the condition shown in figure 9 was found in the majority of the systems dissected from the ovules examined. *P. macrophyllus maki* is similar when two binucleate cells are present, and it agrees with respect to secondary embryos that may be formed on the ends of isolated cells of their prosuspenders.

In *P. totarra*, which usually has a single binucleate embryonic cell, there is normally no cleavage polyembryony from the terminal embryonic portion of the system, and although the prosuspensor cells may break away from the tip as they do in *P. urbanii* and *P. macrophyllus maki*, they do not seem to form embryos

at their tips. The *P. totarra* type has attained simple polyembryony as the usual condition, and only when two binucleate cells are present would it show cleavage polyembryony.

The question as to whether cleavage polyembryony is primitive or advanced may possibly be open to question. DOYLE and LOOBY (10) have concluded, possibly rather hastily, that cleavage polyembryony arose from simple polyembryony in the families in which it occurs, and is therefore more advanced. It is both primitive and advanced in the sense that this condition is found throughout the Coniferales, among members of the most primitive as well as the most advanced families. It is found even in the Gnetales. The writer has contended that it may have had a single origin near the beginning of the evolution of Coniferales, and that the change to simple polyembryony occurred in isolated genera or groups of genera. Cleavage polyembryony is so widespread in this order that—out of thirty-five genera in which conditions are known—only nine genera and parts of two others have simple polyembryony. The conditions in eight genera are not known.

DOYLE and LOOBY's view would necessarily be that cleavage polyembryony originated from simple polyembryony independently in six of the seven families recognized by PILGER (20); here in the Podocarpaceae it would have arisen at least twice. However, the relationships between these families would not be affected by either theory. So far as embryogeny is concerned, these relationships are indicated by the number of free nuclei in the proembryo before walls appear (whether 32, 16, 8, 4, or 2), by the organization of the early embryonic cells, and by the presence or absence of a prosuspensor or primary suspensor, etc. It must be borne in mind, therefore, that a difference of opinion concerning the status of cleavage polyembryony would not affect the relationships between the major groups of this order, but rather the question as to the relationships of genera within the families or subfamilies.

According to my view, simple polyembryony is attained in at least two directions leading away from the types shown in the centrally placed embryos of the series shown in figures 51 and 52. Passing toward the left (fig. 51), simple polyembryony is attained with a maximum number of binucleate cells arranged in four tiers; passing toward the right (fig. 52), it is attained through reduction in the number of binucleate cells which become arranged in a single tier and finally results in a single binucleate cell.

If one were to choose among the embryogenies represented in the series of early embryos shown in figures 51 and 52 a form nearest the prototype from which the Podocarpaceae have had their origin, neither of the extremes would be selected. The prototype was probably most nearly like one of the intermediate forms, such as *P. usambarensis* and *P. gracilior*. A prototype should have thirty-two free nuclei in the proembryo before walls are formed and nine or more binucleate em-

bryonic cells arranged as in these species of *Podocarpus*. The arrangement found in *Saxegothaea*, *Phyllocladus*, and *P. spicatus* would result from the development of the extremely long and narrow archegonia found here, with a reduction of free nuclei to sixteen, while the types found in the direction of *P. totarra* would result from a similar reduction in the number of free nuclear divisions, also affecting the number of embryonic initials. The number of cells in the prosuspensor appears to have been reduced in both directions. The series of embryonic types shown in figures 51 and 52 may serve as a guide to the true relationships within this family.

Taxonomists who may contemplate a monographic treatment of this family should search for external criteria, correlated with the series shown by embryogeny, as a guide to an improved scheme of phylogeny.

Summary and conclusions

1. This investigation is concerned chiefly with details of early stages in the embryogeny of *Podocarpus dacrydioides* and with a more complete series of stages of *P. urbanii*, *P. macrophyllus maki*, *P. totarra*, and *Phyllocladus alpina*. About a dozen additional species of *Podocarpus* were described from observations of one or a few critical stages each. Even though fragmentary, these observations represent critical stages closely comparable with known stages of the species studied more intensively.

2. The embryos of several species belonging to groups not otherwise available for study were obtained, with satisfactory results, from dried herbarium material. In some of these the successive stages could be recognized by comparison with closely related species having the same general type of embryogeny.

3. A summary of comparable stages in the early embryos of the Podocarpaceae was prepared by drawing upon previous investigations. This summary embraces information concerning the embryogeny of about twenty-two species of the Podocarpaceae belonging to four sections or subgenera of *Podocarpus* and to three other genera. It is highly probable that the extremes in the range of variation within this family are represented in this survey.

4. The genus *Podocarpus* is exceptional among conifers in that it includes several more or less distinct types of embryogeny, whereas other genera usually follow a uniform program.

5. Without exception, all species of the Podocarpaceae thus far examined have binucleate embryonic cells. The number of these ranges from one to about twelve. When there are 9-12 binucleate cells in the proembryo, these may be in a single tier, in two tiers, or in three or four tiers; when the number of binucleate cells is five or less they are usually in a single tier. Apparently the binucleate cells are so organized in the proembryo; they do not increase in number after the prosuspensor begins to elongate.

6. The pair of nuclei in an embryonic cell divide simultaneously or nearly so, giving rise to a condition with four telophasic nuclei (sometimes in tetrahedral position), after which walls appear between all nuclei. Thus the binucleate stage passes directly into a 4-celled condition.

7. An apical cell stage may exist for a period of development in some species of *Podocarpus*. In *Phyllocladus*, however, this stage is very brief or may not appear at all; but after the first appearance of walls in the binucleate cells, an appearance of an apical cell is usually simulated.

8. All the Podocarpaceae thus far examined have prosuspensors. Apparently no primary suspensors are formed, but the embryos that are sometimes cut off from the ends of isolated prosuspensor cells may have the appearance of embryos borne on primary suspensors. However, the latter do not pass through a binucleate stage.

9. The number of cells in the prosuspensor varies from 3-4 in *Saxegothaea* (10) and 4-6 in *Phyllocladus* to about eighteen, twenty, or more in *Podocarpus usambarensis* (5), *P. gracilior*, and the *Nageia* group. In the section *Protopodocarpus* the number of such cells varies from 12-14 in *P. macrophyllus maki* and *P. urbanii* to 7-8 in *P. totarra*.

10. The species having twenty or more cells in the prosuspensor, bearing 7-12 binucleate embryo initials, essentially in a single tier, have indeterminate cleavage polyembryony. The species with 7-11 cells in the prosuspensor, bearing 3-7 binucleate embryo initials in one or two tiers, and those with 12-15 cells in the prosuspensor and 2-3 binucleate embryo initials, usually have determinate cleavage polyembryony. The species having about nine or less cells in the prosuspensor, bearing a single binucleate embryonic cell, or those bearing 7-12 binucleate cells in three or more tiers, usually have simple polyembryony.

11. In the later stages of the embryo systems of species with massive prosuspensors, some of the cells were found to have formed embryonic tissue consisting of internal rows of cells. Rosette cells were observed as isolated cells, sometimes giving rise to small rosette embryos.

12. The behavior of some isolated prosuspensor cells and of some rosette cells, when these are found, suggests that not only the binucleate embryonic cells but all cells of the proembryo are potential embryo initials. In the elongating cells of the prosuspensor the embryo-forming capacity is suppressed.

13. The number of free nuclei, before walls appear in the proembryo, is reflected in the number of cells in the proembryo when the prosuspensor begins to elongate. Although the proembryos have actually been observed in detail in only five species of this family, the type of proembryo (for most of them) may be recognized in its general program of development. From these facts and the proembryos which have actually been observed (8, 10, 22, 26), it appears that the number of free nuclei before walls are formed is 16-32.

14. In the organization of cells formed in the early embryo of *P. usambarensis*, *P. gracilior*, and the *Nageia* group of *Podocarpus*, these forms approach closer than any other conifer to the type of cellular organization found in embryos of the *Araucariaceae*. The podocarps differ, however, in having a smaller number of cells, in the fact that the embryonic cells are binucleate, and in giving rise to embryo systems with cleavage polyembryony.

15. The embryogenies of the *Podocarpaceae*, especially within the genus *Podocarpus*, hold great promise—when more completely investigated—of providing the basis for improving the natural system of classification in a taxonomic treatment of this group.

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LITERATURE CITED

1. BUCHHOLZ, J. T., The embryogeny of the conifers. *Proc. Internat. Cong. Plant Sci.* 1:350-392. 1929.
2. ———, The suspensor of *Sciadopitys*. *BOT. GAZ.* 92:243-262. 1931.
3. ———, Determinate cleavage polyembryony, with special reference to *Dacrydium*. *BOT. GAZ.* 94:579-588. 1933.
4. ———, Embryogeny of species of *Podocarpus* of the subgenus *Stachycarpus*. *BOT. GAZ.* 98:135-146. 1936.
5. ———, The dissection, staining, and mounting of the embryos of conifers. *Stain. Technol.* 13:53-64. 1938.
6. BUCHHOLZ, J. T., and OLD, EDNA M., The anatomy of the embryo of *Cedrus* in the dormant stage. *Amer. Jour. Bot.* 20:35-44. 1933.
7. BURLINGAME, L. L., The morphology of *Araucaria braziliensis*. III. Fertilization, the embryo, and the seed. *BOT. GAZ.* 59:1-39. 1915.
8. COKER, W. C., Notes on the gametophyte and embryo of *Podocarpus*. *BOT. GAZ.* 33:89-107. 1902.
9. COULTER, J. M., and CHAMBERLAIN, C. J., *Morphology of gymnosperms*. Chicago. 1910.
10. DOYLE, J., and LOOBY, W. J., Embryogeny in *Saxegothaea* and its relation to other podocarps. *Sci. Proc. Roy. Soc. Dublin* 22:127-147. 1930.
11. EAMES, A. J., The morphology of *Agathis australis*. *Ann. Bot.* 27:1-38. 1913.
12. GIBBS, L. S., On the development of the female strobilus in podocarps. *Ann. Bot.* 26:515-572. 1912.
13. HOLLOWAY, J. T., Ovule anatomy and development and embryogeny in *Phyllocladus alpinus* (Hook.) and in *P. glaucus* (Carr.). *Trans. Proc. Roy. Soc. New Zealand* 67:149-165. 1937.
14. KILDAHL, N. JOHANNA, The morphology of *Phyllocladus*. *BOT. GAZ.* 46:339-348. 1908.
15. LAWSON, A. A., The gametophytes and embryo of *Sciadopitys verticillata*. *Ann. Bot.* 24:403-421. 1910.
16. ———, The life history of *Microcachrys tetragona* Hook. *Proc. Linn. Soc. New South Wales*. Part 4. 48:499-514. 1923.
17. LOOBY, W. J., and DOYLE, J., The ovule, gametophytes, and proembryo in *Saxegothaea*. *Sci. Proc. Roy. Soc. Dublin* 22:95-117. 1930.

18. MIRBEL, and SPACH, Notes sur l'embrogenie des *Pinus laricio* et *sylvestris*, des *Thuja orientalis* et *occidentalis* et du *Taxus baccata*. Ann. Sci. Nat. Bot. II (20): 257-268. 1843.
19. PILGER, R., Taxaceae in ENGLER, Pflanzenreich IV. 5. Leipzig. 1903.
20. ———, Coniferae in ENGLER, Nat. Pflanzenfamilien (Podocarpaceae). 13:211-249. 1926.
21. SAHNI, B., The structure and affinities of *Acmopyle pancheri* Pilger. Phil. Trans. Roy. Soc. London B 210:253-310. 1920.
22. SINNOTT, E. W., The morphology of the reproductive structures in the Podocarpaceae. Ann. Bot. 27:39-82. 1913.
23. STILES, W., The Podocarpaceae. Ann. Bot. 26:443-514. 1912.
24. STRASBURGER, E., Die Angiospermen u. die Gymnospermen. Jena. 1879.
25. TAHARA, MASATO, Contributions to the morphology of *Sciadopitys verticillata*. Cytologia, Fujii Jubilee Vol. (1937):14-19. 1937.
26. ———, Embryogeny of *Podocarpus macrophyllus* and *Podocarpus nagi*. Science Reports, Tokohu Imperial University Ser. iv. (Biology) 14:91-98. 1941.
27. YOUNG, MARY S., The morphology of the Podocarpaceae. BOT. GAZ. 50:81-100. 1910.

SEASONAL CHANGES IN BOUND WATER CONTENT OF SOME PRAIRIE GRASSES

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(WITH FOUR FIGURES)

Introduction

In a study of certain grassland vegetation types in western North Dakota, it seemed desirable to attempt to use the bound water content of some of the principal grass species of the various types as a measure of the adaptation of the species to their respective habitats. In this semi-arid region the amount of moisture available for use by the plants is in most cases the principal factor governing the occurrence of different vegetation types. Consequently the measurement of a property which might be an integrated expression of the relation of the plant to its habitat, especially to the available soil moisture, could be expected to throw considerable light on the essential relations between species within the vegetation types and between the types themselves.

This investigation involved weekly determinations of the bound water content of the leaf tissue of seven grass species in four different grassland types during the summer seasons of 1937 and 1938. By bound water is meant that water which remained unfrozen in the leaf tissue after a freezing period of 6 hours at -21.5°C . Various investigators have referred to this water as "unfrozen" (22) or as "un-freezable" (5, 27).

Review of literature

SPOEHR (20) found that when certain species of *Opuntia* were subjected to continued periods of drought the pentosan content of the tissues showed decided increase. It was concluded that the plants were enabled to absorb and retain large quantities of water because of the presence of these hydrophilic colloids of high imbibition capacity. MAXIMOV (8) states that in both frost and drought resistance the basis of the resistance lies in the capacity of the protoplasm to withstand the dehydrating influence of a direct or indirect deprivation of water.

NEWTON and MARTIN (14) were apparently the first to investigate the relations between drought tolerance, colloidal content, and bound water in plant tissues. They made an extensive study of bound water in relation to drought resistance in crop plants and in native and tame grasses, using the cryoscopic technique of NEWTON and GORTNER (13). They found that in southern Alberta the percentage

¹ Mr. ROALD PETERSON and Mr. H. THEO HANSON assisted in the field work of this study.

bound water content of the expressed sap of some of the native grasses correlated directly with the known adaptation of these species to drought conditions. In their studies bound water determinations were made during two seasons, 1925 and 1926.

LEBEDINCEV (7) found that xerophytes growing in a semi-desert region had a higher water-retaining capacity than had mesophytes growing under the same conditions. After repeated wiltings there was a permanent increase in their water-retaining capacity. She concluded, however, that the main role in determining this capacity was played by osmotic substances and not by colloids. In contradiction to this, VASSILIEV and VASSILIEV (25) found that in young wheat plants exposed to drought the hemicelluloses increased and remained high even after a return to normal water supply. The hemicelluloses are of the pentosan group and have marked capacity to swell. BARINOVA (1) also found in the case of sugar beets that an impoverishment of water in the tissues resulted in an increase in hydrophilic colloids in the leaves and roots.

NOVIKOV (15) found that the quantity of bound water in winter wheats, determined when the plants were undergoing drought, was a measure of the drought resistance of the variety. When the plants were growing under optimum moisture conditions, however, they showed no essential differences in water-retaining capacity. He concluded that the increase in this capacity during drought hardening resulted from an increase in water-retaining substances in the plants and not from losses of free water from the plant tissue.

CALVERT (3) studied drought resistance in three varieties of wheat. He determined the total water, percentage free water, percentage bound water, and total solids in the sap expressed from the leaves. The determinations were made at frequent intervals throughout the season, and soil moisture determinations were also made. He found that there was a slight decrease in the percentage of total water in the sap from the beginning to the end of the season, but on the whole the total water remained fairly constant. The percentage of free water in the sap, however, decreased throughout the season. This decline was compensated by a corresponding increase in bound water. This increase was real, whether expressed on the basis of percentage total water or per gram of dry material in the sap. He concluded that the external conditions which reduce the free water per gram of dry material act simultaneously on the plant in such a way as to increase the bound water per gram of dry matter.

In a later study on the sap expressed from the leaves of *Phalaris tuberosa*, CALVERT (2) found that—per gram of dry matter—the total water, the free water, and the bound water were all significantly greater in the morning than in the afternoon. The decrease in free water in the afternoon was considerably greater than the decrease in bound water, and at that time there was actually more bound

water per gram of dry matter than there was free water. These reversed relations apparently resulted from the water deficit created by the increase in transpiration during the day. This work indicates that the bound water content of a species may be directly influenced by the varying conditions of the environment.

In a study of water relations in detached drying leaves of *Bryophyllum calycinum*, WELCH (27) found that as the leaf dried the percentage of "unfreezable" or bound water increased, while the percentage of "freezable" or free water decreased. The increase in bound water did not seem to result from an increase in the water-retaining capacity of the leaf tissues, but rather increased merely as the result of loss of free water in the drying process.

SCHOPMEYER (19) studied transpiration and physico-chemical properties of leaves of loblolly and shortleaf pine in relation to drought resistance. He found that the more drought-resistant shortleaf pine had only slightly more bound water than loblolly pine when the soil moisture was at the wilting coefficient; and in the other cases when soil moisture was at an optimum value both before and after wilting it had less bound water, even though the total moisture content of this species was greater at all times than that of loblolly pine. He concluded that the greater drought resistance of shortleaf pine could not be attributed to an ability to conserve water either by retarding transpiration or by forming bound water.

Material and methods

Bound water in the leaf tissue was determined by the calorimetric or heat-of-fusion method. The technique followed was essentially similar to that described by GREATHOUSE (5). The method was originally used by MÜLLER-THURGAU (12) and elaborated by RÜBNER (17), and later by THOENES (24). THOENES (24), ROBINSON (16), ST. JOHN (23), MEYER (11), SAYRE (18), STARK (21), CHRYSLER (4), GREATHOUSE (5), and WELCH (27) all give excellent discussions of the use of the calorimetric method and the calculation of bound water from calorimetric data.

A sample of leaf tissue weighing approximately 10 gm. was rapidly cut into short lengths and placed in a weighed tinfoil cup. A small lead weight was placed in the cup with the tissue, and the top of the container was folded over and pressed shut. The sample was then weighed and put into a large test tube, which was in turn placed in an acetone-solid carbon dioxide freezing bath, where it was frozen at $-21.5^{\circ} \pm 0.5^{\circ}$ C. for 6 hours. Samples were run in duplicate. At the end of about 3 hours the samples were removed from the bath and the containers rapidly slit with a sharp knife. The samples were then returned to the tubes and to the freezing bath. This treatment assured the more rapid establishment of an equilibrium temperature in the calorimeter.

The amount of water frozen in the plant tissues during the freezing period was measured by introducing the sample into a calorimeter containing exactly 250 gm.

of water at slightly above room temperature. The water in the calorimeter was stirred rapidly until an equilibrium temperature was reached. The change in temperature of the water was measured by means of a mercury thermometer graduated to 0.05° F. With the aid of a lens, the reading could be accurately estimated to 0.01° F.

The calorimeter consisted of a pint Dewar flask held in a small wooden case. A space approximately 1 inch wide between the flask and the edges of the case was entirely filled with rock wool, thoroughly insulating the flask. A base of heavy wood supported the case, which fitted closely against the sides and back of the base and was thus held firmly in place. Two vertical steel rods, one on each side of the base, supported the carrying platform, to which motor, stirrer, thermometer, and stopper were attached. The platform was adjusted to move up and down on the steel rods, and it could be locked in any position. This arrangement greatly facilitated the opening and closing of the calorimeter.

A spiral glass stirrer driven by a small electric motor was used to stir the water in the calorimeter. The time required to obtain equilibrium with a 10-gm. sample was about 6-8 minutes.

The water which froze in the samples (free water) was calculated according to the formula given by GREATHOUSE (5), with the exception that the use of a lead weight in the samples to insure their complete submergence necessitated the inclusion of an additional factor in the formula. The calorimeter factor, as determined from repeated trials with 10 gm. of water in the tinfoil cup instead of a tissue sample, was 1.094.

The bound water was determined by subtracting the weight of the water frozen from the total water in the sample:

$$W_b = W_w - W_i$$

The total water was secured by subtracting the weight of the dried sample from the green weight. The dry weight and the specific heat of the tissue sample were obtained with the same sample used in the determination of the free water. The samples were dried to constant weight at 88° C. Following the procedure of MEYER (11), CHRYSLER (4), and others, the specific heat determinations were made on the dried tissue, using benzene in the calorimeter.

The osmotic value of the plant sap was calculated from the depression of the freezing point. The heating method (10) was used to kill the tissue. The sap was expressed from the leaf tissue at a pressure of 15,000 pounds per square inch, and the freezing point was determined with the Drucker-Burian microthermometer. The use of this apparatus was originally described by WALTER (26). MALLERY (9) gives a complete discussion of the method and points out that it is especially

adapted for use in arid regions, since only 1.5-2 ml. of plant sap is necessary in order to make a freezing point determination.

Tissue samples for the bound water and osmotic values were collected in the early morning from the various vegetation types. Each of the four areas was sampled once each week, two being sampled on one day and the other two on the following day. The samples in each type were collected from the area encompassed by a circle with a radius of approximately 50 feet, the point of sampling being at the approximate center of the circle. These samples were also taken once each week.

The tissue samples were placed in pint Mason jars equipped with screw or clamp tops and rubber sealing rings. The jars were then put in a heavy cardboard box and taken back to the laboratory. Samples which could not be handled immediately were placed in the refrigerator. None of the tissue samples was kept for more than 3 hours before preparation, and usually work was begun on them within 15-20 minutes after they reached the laboratory.

Vegetation types

This work was conducted at the Pyramid Park Range Station, located 17 miles southwest of Fryburg, North Dakota, in the broken range country of the Badlands. Four areas, none of which was more than 4 miles from the station, were selected as sample areas. These areas were typical representatives of four of the principal grassland types.

1. UPLANDS TYPE.—One of the areas was on a nearly level uplands terrace 4 miles south of the range station (S.W. $\frac{1}{4}$ sec. 16-138-101). The vegetation was typically that of the grama-needlegrass-sedge type (6). The principal species were blue grama grass, *Bouteloua gracilis* H. B. K. Lag.; needle-and-thread, *Stipa comata* Trin. & Rupr.; niggerwool, *Carex filifolia* Nutt.; and western wheatgrass, *Agropyron smithii* Rydb. The species selected for sampling in this area were grama grass, needle-and-thread, and western wheatgrass.

The supply of soil moisture in the uplands type is dependent upon the precipitation received during the season. The subsoil is perpetually dry. In ordinary seasons there is usually no available moisture below 2 feet, and moisture in the upper levels is usually deficient by the middle of July. This is the most xerophytic of the grassland types included in this study.

2. SAGEBRUSH TYPE.—The second sampling area was in the flats along streams and in valleys; it is characterized by the dominance of the gray-colored shrub, *Artemisia cana* Pursh., growing in dense or open stands. This second area was in a typical development of the type found on a low terrace along Davis Creek, about $\frac{1}{2}$ mile from the station (N.E. $\frac{1}{4}$ sec. 11-138-101). The principal grass species are western wheatgrass, grama grass, needle-and-thread, and green needlegrass, *Stipa*

viridula Trin. The same three species were sampled here as in the uplands type—western wheatgrass, grama grass, and needle-and-thread.

The fact that the same species are abundant in the sagebrush type as in the uplands type indicates that there is considerable similarity between the habitats. There are, however, two important differences. The sagebrush type, situated as it is on a terrace flat along a creek bed, has a supply of subsoil moisture, at least for a part of the season. For the most part this moisture is out of reach of the roots of the grasses, but it is tapped by the extensive root system of the sagebrush. In addition, although Davis Creek is dry for most of the season, occasional torrential rains cause the creek to flood over its banks and inundate the terrace on which the sample area was situated. Thus some additional moisture may be received during the season.

3. SANDGRASS TYPE.—The third area selected was on a low sandy ridge about $\frac{1}{4}$ mile west of the uplands type (S.E. $\frac{1}{4}$ sec. 16-138-101). The principal dominant in this type is sandgrass, *Calamovilfa longifolia* (Hook.) Scribn. Important secondary species include the carices *Carex filifolia* Nutt., *C. eleocharis* Wahl., and *C. pennsylvanica* Lam., as well as the grasses, grama grass, needle-and-thread, and Junegrass, *Koeleria cristata* (L.) Pers. The species sampled in this type were sandgrass and Junegrass.

The soil of the sandgrass type is sandy, averaging about 75 per cent sand to a depth of 5 feet. Moisture storage in this type is rather deep because of the coarse nature of the soil material. During most of the season there is an appreciable amount of moisture in the third foot, frequently more than in the upper 2 feet, and presumably the moisture supply is even greater below 3 feet. Unfortunately, records of soil moisture are available only to a depth of 2 feet for the seasons during which the determinations were made.

4. BIG BLUESTEM TYPE.—This type is developed on the lower parts of steep slopes, such as occur in the narrow valleys and draws of the region. It represents the most mesophytic grassland in the region and is really an extension of the eastern tall grass prairie into semi-arid western North Dakota. Vegetation and soil show a high degree of development, which apparently is dependent on receiving more moisture than that furnished by direct precipitation. The additional moisture is received from seepage, runoff, and melting snow drifts. The area selected for sampling was located about 3 miles northwest of the station, on the steep east-facing slope of a narrow valley (N.E. $\frac{1}{4}$ sec. 3-138-101). Big bluestem, *Andropogon furcatus* Muhl.; porcupine grass, *Stipa spartea* Trin.; and side-oats grama, *Bouteloua curtipendula* (Michx.) Torr., are the principal species occurring in this type. Big bluestem and porcupine grass were the species considered in this study.

Experimental results

1. UPLANDS TYPE.—Tables 1 and 2 and figure 1 present the data for the 1937 and 1938 seasons in the uplands type. The outstanding features of the behavior of the species in this type are the marked increases in bound water as percentage of

TABLE 1
MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER
IN SPECIES IN UPLANDS TYPE; 1937 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O% TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O% TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
AGROPYRON SMITHII							
6/28.....		63.0	16.91	20.90	35.56	79.10	134.55
7/3.....	11.1	62.1	15.60	27.86	45.70	72.14	118.33
7/12.....	11.8	56.8	17.76	31.09	40.06	68.91	90.70
7/19.....	9.2	52.1	19.37	35.54	38.64	64.46	70.08
7/26.....	9.6	45.7	22.97	46.76	39.40	53.24	44.86
8/2.....							
STIPA COMATA							
6/28.....		55.8	15.44	31.24	39.46	68.76	86.92
7/3.....	11.1	54.9	14.43	34.29	41.72	65.71	79.91
7/12.....	11.8	50.5	15.48	36.16	36.84	63.84	65.04
7/19.....	9.2	46.7	18.20	37.93	33.22	62.07	54.37
7/26.....	9.6	34.3	31.00	62.18	32.45	37.82	19.74
8/2.....	7.8	28.4	33.48	80.90	32.12	19.10	7.58
BOUTELOUA GRACILIS							
6/28.....		64.4	13.55	29.71	53.63	70.29	126.90
7/3.....	11.1	61.8	13.65	29.43	47.57	70.57	114.08
7/12.....	11.8	59.6	13.82	34.38	50.72	65.62	96.81
7/19.....	9.2	58.8	16.05	35.27	50.26	64.73	92.23
7/26.....	9.6	43.5	26.44	66.26	50.93	33.74	25.94
8/2.....	7.8	26.9	19.56	98.34	36.21	1.66	0.61

* Average wilting coefficient to depth of 3 feet, 12.5%.

total water and the marked decreases in free water percentage, in grams of free water per 100 gm. of dry matter, and in percentage of moisture in the tissue of the different species. Osmotic values show considerable increase. The values of grams of bound water per 100 gm. of dry matter apparently show no general constant tendencies, although the 1938 results indicate a seasonal increase in this value for two of the species.

As shown in figure 1, the decrease in soil moisture during the course of the season was approximately paralleled by a decrease in the percentage of moisture in the tissue. These decreases in total water in the tissue are accompanied by decreases in the percentage free water and in the grams of free water per 100 gm. of dry

TABLE 2
MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER
IN SPECIES IN UPLANDS TYPE; 1938 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
AGROPYRON SMITHII							
6/28.....	11.8	62.5	13.40	21.41	35.70	78.59	131.06
7/2.....	15.7	59.8	12.74	26.12	38.89	73.88	109.81
7/8.....	13.6	57.3	13.56	24.48	32.91	75.52	101.47
7/17.....	9.7	51.2	16.28	32.55	35.59	67.47	73.80
7/25.....	8.5	53.2	17.57	33.17	37.70	66.83	75.89
8/2.....	7.0	40.7	22.41	48.34	33.23	51.66	35.50
8/6.....	6.7	41.8	20.52	41.89	30.11	58.11	41.70
STIPA COMATA							
6/26.....	11.8	58.4	12.64	16.23	22.74	83.77	117.50
7/2.....	15.7	55.1	11.69	21.89	26.94	78.11	95.99
7/8.....	13.6	52.2	12.65	28.29	30.94	71.71	78.57
7/17.....	9.7	42.8	16.86	37.77	28.33	62.23	46.68
7/25.....	8.5	42.7	22.10	47.21	35.18	52.79	39.32
8/2.....	7.0	30.9	29.05	66.34	29.50	33.66	10.34
8/6.....	6.7	35.9	31.08	50.19	28.15	49.81	27.93
BOUTELOUA GRACILIS							
6/26.....	11.8	65.2	8.34	15.51	29.10	84.49	158.50
7/2.....	15.7	63.8	8.14	19.05	33.60	80.95	142.74
7/8.....	13.6	60.9	8.29	25.71	40.03	74.29	115.64
7/17.....	9.7	50.4	13.07	39.57	40.09	60.43	61.82
7/25.....	8.5	49.3	19.32	47.39	45.93	52.61	51.21
8/2.....	7.0	36.0	24.11	74.03	41.37	25.97	15.07
8/6.....	6.7	32.8	27.16	92.92	45.41	7.08	3.14

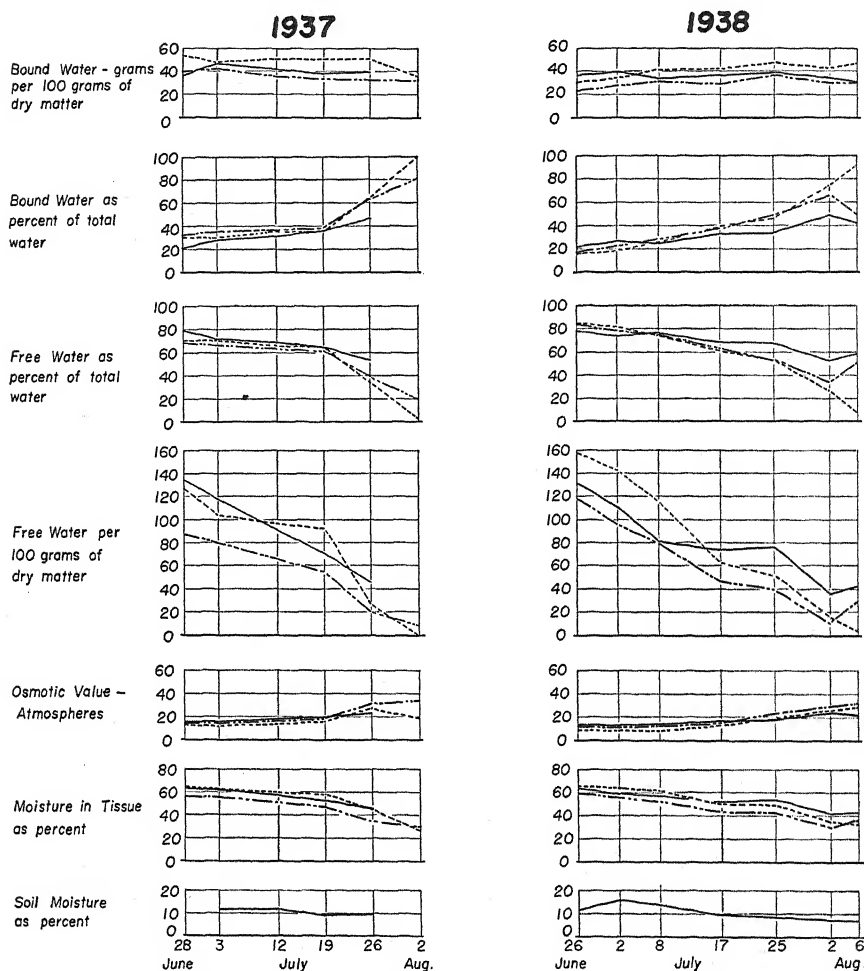
* Average wilting coefficient to depth of 3 feet, 12.5%.

matter. The grams of bound water per 100 gm. of dry matter do not show any drastic increases or decreases. At the same time values for percentage of bound water increase remarkably.

The outstanding behavior among the individual species is shown by grama

grass. In 1937 the bound water as percentage of total water reached the value of 98.3 by August 2. At this time there was approximately 37 gm. of water per 100

UPLANDS TYPE



Agropyron smithii ————— *Bouteloua gracilis* - - - - - *Stipa comata* -

FIG. 1.—Changes in bound and free water, osmotic value, and total moisture in leaf tissue of western wheatgrass, blue grama grass, and needle-and-thread in uplands type during summer seasons of 1937 and 1938. Soil moisture also given.

gm. of dry matter in the leaf tissue. In 1938 this same species contained 92.9 per cent bound water on August 6, and the total water content was 48.5 gm. per 100 gm. of dry matter. None of the other species in any of the types equaled these

TABLE 3

SEASONAL AVERAGES OF OSMOTIC VALUE AND BOUND AND FREE WATER FOR ALL SPECIES AND TYPES

TYPE	SPECIES	1937 SEASON				1938 SEASON					
		OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GM./100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GM./100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GM./100 GM. DRY MATTER	
Uplands	<i>Agropyron smithii</i>	18.52	13.47	40.05	67.57	91.72	16.64	32.56	34.88	67.44	81.32
	<i>Bouteloua gracilis</i>	17.18	48.90	48.22	51.10	76.09	15.49	44.82	39.36	55.12	78.30
	<i>Stipa comata</i>	21.34	47.12	35.97	52.88	52.26	19.44	38.27	28.83	61.73	59.48
	All species.....	19.01	42.83	41.41	57.18	73.36	17.19	38.55	34.30	61.43	73.03
Sagebrush	<i>Agropyron smithii</i>	25.30	28.88	36.33	71.12	101.20	19.57	29.73	34.22	70.27	97.48
	<i>Bouteloua gracilis</i>	24.50	42.80	49.95	57.11	60.80	17.00	50.58	41.92	49.50	61.10
	<i>Stipa comata</i>	25.05	37.85	38.54	62.15	68.11	27.63	39.97	31.71	60.03	59.22
	All species.....	24.95	36.54	41.61	63.46	79.70	21.40	40.09	35.95	59.95	72.60
Sandgrass	<i>Calamovilfa longifolia</i> ...	11.76	32.02	41.79	67.98	88.55	10.73	27.16	36.69	72.84	100.78
	<i>Koeleria cristata</i>	15.84	35.23	43.65	64.77	88.91	15.67	31.60	36.73	64.80	86.48
	All species.....	13.80	33.62	42.72	66.37	88.73	13.20	29.38	36.71	70.62	93.63
Big blue- stem	<i>Andropogon furcatus</i>	9.11	25.69	51.76	74.30	145.30	9.42	22.38	48.94	77.62	170.95
	<i>Stipa spartea</i>	13.13	27.55	32.74	72.45	86.80	14.36	24.27	29.97	75.73	94.10
	All species.....	11.12	26.62	42.25	73.37	116.05	11.89	23.32	39.45	76.67	132.52

values. In both seasons the bound water per 100 gm. of dry matter was higher in this than in any of the other species in this type. This is shown in figure 1 and in table 3, where the seasonal averages for all species and types are given.

Needle-and-thread was second highest in both seasons in bound water as percentage of total water, while western wheatgrass was the lowest of the three species in this value. In contrast to this, needle-and-thread was lower in grams of bound water per 100 gm. of dry matter at nearly all dates of sampling in both seasons than was western wheatgrass. Needle-and-thread was also lower in grams of free water per 100 gm. of dry matter throughout most of the season. On the basis of the averages for the grams of free water (table 3), western wheatgrass ranked highest and grama grass second. These two species were also higher in total water content than was needle-and-thread.

Osmotic values were fairly uniform in all three species during the seasons. Needle-and-thread, with the lowest total water content and the lowest seasonal values for grams of free water per 100 gm. of dry matter, reached the highest osmotic values at the end of the season. This would indicate that the osmotic effects were not the controlling factor in determining bound water content, since western wheatgrass and grama grass, both with lower average osmotic values than needle-and-thread, were higher in bound water expressed either as percentage or on the dry-weight basis.

In the uplands type during the two seasons the effects of decreasing soil moisture became evident almost from the beginning of the sampling period. By shortly after mid-July the moisture situation became acute, and in both years the species became too dry to sample by the end of the first week in August. Bound water and osmotic values showed their greatest increases after the soil moisture dropped below an approximate 10 per cent level.

2. SAGEBRUSH TYPE.—The data presented in tables 4 and 5 and in figure 2 indicate that there is considerable similarity in behavior of the species sampled in the sagebrush and in the uplands type. The more uniform results obtained for the species in the sagebrush type in 1938 show a greater similarity to the results obtained for the same species in the uplands type than do the 1937 results. In general, the same marked increases in bound water as percentage of total water and the marked decreases in percentage free water, grams of free water per 100 gm. of dry matter, and percentage of moisture in the tissue were shown by these species. With the exception of the behavior of grama grass in 1937, the values of grams of bound water per 100 gm. of dry matter were fairly uniform in the species during both seasons.

Grama grass again had the highest values for bound water as percentage of total water, and in 1938 a maximum of 92.1 per cent for this value was observed on August 2. In table 3 it is shown that the averages for percentage bound water were

higher in this species during both seasons than in any of the other types. As in the uplands type, needle-and-thread was second highest in percentage bound water, while western wheatgrass again had lower average values for this factor than either of the other species.

Grams of bound water per 100 gm. of dry matter was higher in grama grass than in the other species, but in the 1937 season needle-and-thread averaged below western wheatgrass in regard to this value, although averaging higher in the 1938 season. In the uplands type, values for grams of bound water averaged higher during both seasons in needle-and-thread than they did in western wheatgrass.

Figure 2 and tables 4 and 5 show that in the sagebrush type, grama grass was lower in percentage of free water and in grams of free water per 100 gm. of dry matter throughout most of the sampling period than were needle-and-thread and western wheatgrass. Needle-and-thread was intermediate between grama grass and western wheatgrass in free water per 100 gm. of dry matter for most of the season. The free water in all species in both uplands and sagebrush types decreased at a rather rapid rate from the beginning of the sampling period to its end.

The osmotic values of all species in the type showed a general increasing trend during the season. The average osmotic values in the species in the sagebrush type were higher than the average values of the species in the uplands type. The total moisture in the tissue decreased in all species in both types, the rate and extent of decrease being similar during comparable seasons.

Average soil moisture to a depth of 2 feet in the sagebrush type was not once above the calculated wilting coefficient of 9.2 per cent during the sampling period in 1937. In the 1938 season the average soil moisture was above the calculated wilting coefficient until about the middle of July, and in this season the marked decreases in percentage of moisture in the tissue and in percentage of free water began at about this time.

3. SANDGRASS TYPE.—The species sampled in this type showed a behavior different from those sampled in the uplands and sagebrush types. The marked increases in percentage bound water with the progress of the season were not observed in Junegrass or in sandgrass. Junegrass showed relatively greater changes in percentage bound water, osmotic value, total moisture, and in percentage and grams of free water than did sandgrass. Yet in general the range of the values was considerably smaller than the range of these same values in the species in the sagebrush and upland types (tables 6, 7; fig. 3). They appear to be primarily related to moisture conditions and to the type of root systems of the species sampled in the sandgrass type. The sandy soil of the sandgrass area permits relatively deep moisture storage, and the wilting coefficient is lower, being only 7.1 per cent. The moisture in the upper 2 feet of the soil was below this value from the middle

of July to the end of the sampling period during 1937 and in 1938 was not above the wilting coefficient after the end of July.

SAGEBRUSH TYPE

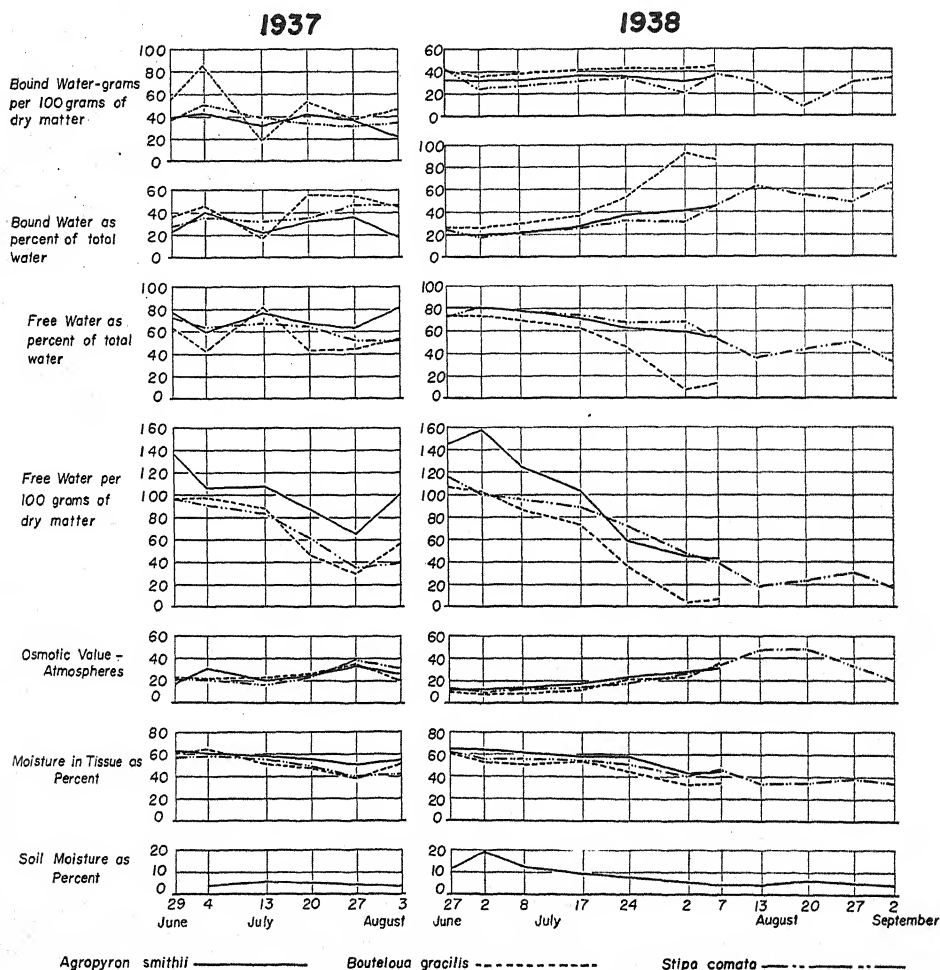


FIG. 2.—Changes in bound and free water, osmotic value, and total moisture in leaf tissues of western wheatgrass, blue grama grass, and needle-and-thread in sagebrush type during summer seasons of 1937 and 1938. Soil moisture also included.

Junegrass, which has a relatively shallow root system, was apparently unable to utilize the deeper moisture, and when moisture in the upper soil levels was largely depleted this species became too dry to sample. The values for percentage bound water, free water, osmotic concentration, and total moisture in the tissue

seem to reflect to considerable extent the soil moisture situation. In the case of sandgrass there was only a slight decrease in the total moisture in the tissue during the sampling period. Increases in the percentage of bound water were small or nonexistent in this species. Values for grams of bound water per 100 gm. of dry

TABLE 4
MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER
IN SPECIES IN SAGEBRUSH TYPE; 1937 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
AGROPYRON SMITHII							
6/29.....		63.6	17.60	22.34	39.58	77.66	137.58
7/4.....	4.0	60.1	31.20	40.92	43.90	59.08	106.45
7/13.....	5.3	58.4	20.15	22.88	32.14	77.12	108.34
7/20.....	5.1	56.6	23.74	32.56	42.40	67.44	87.83
7/27.....	4.1	50.7	32.71	36.44	37.42	63.56	65.27
8/3.....	3.6	55.4	26.38	18.15	22.56	81.85	101.71
STIPA COMATA							
6/29.....		57.4	20.44	27.89	37.56	72.11	97.13
7/4.....	4.0	58.9	20.10	36.03	51.68	63.97	91.76
7/13.....	5.3	55.1	16.18	32.29	39.66	67.71	83.17
7/20.....	5.1	48.9	23.52	35.76	34.28	64.24	61.57
7/27.....	4.1	40.4	38.37	47.36	32.08	52.64	35.65
8/3.....	3.6	43.0	31.71	47.75	35.98	52.25	39.37
BOUTELLOUA GRACILIS							
6/29.....		60.5	22.34	36.14	55.36	63.86	97.83
7/4.....	4.0	64.9	20.21	46.77	86.51	53.23	98.45
7/13.....	5.3	51.8	21.74	17.93	19.26	82.07	88.14
7/20.....	5.1	48.8	25.95	56.32	53.72	43.68	46.28
7/27.....	4.1	40.6	36.65	55.02	37.68	44.98	30.80
8/3.....	3.6	51.1	20.10	45.16	47.17	54.84	57.28

* Average wilting coefficient to depth of 3 feet, 9.2%.

matter in sandgrass fluctuated somewhat but seemed to show no constant significant tendencies (fig. 2). The grams of free water per 100 gm. of dry matter in this species showed a consistent decreasing trend with advance of the season.

Table 3 shows that the average value for percentage bound water of the two species in the sandgrass type was lower than the averages for percentage bound

water of the species in the uplands and sagebrush types. The grams of bound water per 100 gm. of dry matter for the two sandgrass species averaged higher,

TABLE 5
MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER
IN SPECIES IN SAGEBRUSH TYPE; 1938 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
ACROPYRON SMITHII							
6/27.....	11.8	64.0	11.36	18.31	32.65	81.69	145.69
7/2.....	19.3	64.0	12.41	18.32	32.85	81.68	158.43
7/8.....	12.9	61.5	13.55	21.23	33.96	78.75	125.99
7/17.....	9.9	58.6	17.02	26.17	37.12	73.83	104.79
7/24.....	8.0	48.6	23.57	37.73	35.76	62.27	59.04
8/2.....	5.9	43.4	27.63	40.98	31.50	59.02	45.31
8/7.....	4.9	44.0	31.47	45.37	35.69	54.63	43.14
STIPA COMATA							
6/27.....	11.8	61.4	13.35	26.64	42.56	73.36	116.12
7/2.....	19.3	55.7	9.51	19.91	25.06	80.09	100.71
7/8.....	12.9	55.2	12.84	22.24	27.54	77.76	96.18
7/17.....	9.9	54.7	14.76	26.06	31.42	73.94	89.41
7/24.....	8.0	51.8	18.32	32.53	34.90	67.47	72.38
8/2.....	5.9	40.4	26.69	31.47	21.86	68.53	46.18
8/7.....	4.9	45.1	34.01	46.12	38.00	53.88	39.10
8/13.....	4.7	33.3	47.73	63.50	31.82	36.50	18.27
8/20.....	6.1	34.6	48.57	55.14	29.30	44.86	23.84
8/27.....	5.4	38.3	33.80	49.81	30.93	50.19	31.18
9/2.....	4.6	34.8	44.39	66.23	35.43	33.77	18.07
BOUTELOUA GRACILIS							
6/27.....	11.8	61.5	10.60	26.13	41.74	73.87	117.91
7/2.....	19.3	57.9	8.13	26.45	36.47	73.55	101.45
7/8.....	12.9	55.9	9.01	30.75	39.06	69.25	87.95
7/17.....	9.9	53.7	12.27	37.14	43.00	62.86	73.19
7/24.....	8.0	44.2	21.59	53.88	43.61	46.12	36.33
8/2.....	5.9	32.1	23.03	92.06	43.49	7.94	3.75
8/7.....	4.9	34.7	34.35	86.65	46.09	13.35	7.10

* Average wilting coefficient to depth of 3 feet, 9.2%.

however, than this same value for the species mentioned in the previous discussion. The average values for grams of free water per 100 gm. of dry matter also were considerably higher in Junegrass and sandgrass than they were in western

TABLE 6

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN SANDGRASS TYPE; 1937 SEASON

DATE	SOIL MOIS- TURE* (%)	CALAMOVILLEA LONGIFOLIA						KOELERIA CRISTATA					
		MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER	MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
6/28....	61.6	11.80	32.34	51.77	67.66	108.33	65.2	13.89	29.04	54.54	70.96	133.23
7/3.....	6.5	63.5	8.34	34.60	60.25	65.40	113.88	63.9	12.59	29.01	53.06	70.09	124.35
7/12....	7.1	57.2	9.80	27.68	37.08	72.31	90.87	59.2	13.54	23.87	34.69	76.13	110.66
7/19....	5.2	55.8	11.14	38.27	48.52	61.73	78.25	54.1	15.54	35.13	41.41	64.87	76.46
7/26....	4.7	55.5	13.60	30.83	38.40	69.17	86.15	46.5	20.45	49.50	42.99	50.50	43.86
8/2.....	4.1	55.4	11.74	25.10	31.22	74.90	93.17	44.5	19.04	43.95	35.21	56.05	44.91
8/9.....	3.7	52.5	14.25	31.44	34.80	68.56	75.89	42.11
8/16....	3.2	55.0	11.96	20.82	36.50	70.18	85.91
8/23....	3.6	52.5	12.80	36.63	40.46	63.37	70.00
8/30....	3.7	53.7	12.21	33.49	38.78	66.51	77.02

* Average wilting coefficient to depth of 3 feet, 7.1%.

TABLE 7

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN SANDGRASS TYPE; 1938 SEASON

DATE	SOIL MOIS- TURE* (%)	CALAMOVILEA LONGIFOLIA						KOEELERIA CRISTATA					
		MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER	MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
6/26....	11.7	61.6	8.49	20.51	33.10	79.49	127.41	63.9	10.73	25.03	44.36	74.97	132.81
7/2.....	13.6	60.8	7.78	20.71	32.22	79.29	123.25	63.2	10.17	18.61	32.01	81.39	139.88
7/8.....	11.0	60.4	8.27	20.84	32.39	79.16	123.06	61.7	10.29	22.35	36.01	77.05	125.11
7/17....	7.6	59.0	6.90	29.14	41.90	70.86	102.04	55.1	13.79	26.95	33.19	73.95	89.86
7/25....	6.6	59.7	7.93	26.70	39.68	73.30	108.93	59.8	10.82	31.37	46.75	68.63	102.21
8/2.....	5.2	55.1	13.62	25.17	30.88	74.83	91.60	51.0	21.00	35.38	36.88	64.62	67.23
8/6.....	5.0	50.7	9.76	32.19	42.20	67.81	88.77	52.0	12.83	34.16	37.21	65.84	71.80
8/13....	4.7	53.5	12.16	30.71	35.46	69.29	79.96	38.4	21.80	50.08	31.38	49.92	1.38
8/20....	4.6	55.3	21.41	27.35	33.83	72.65	90.28	44.6	28.72	40.44	32.65	59.56	48.09
8/27....	4.4	54.3	10.42	33.05	40.64	66.95	82.29
9/2.....	4.4	55.9	11.31	32.40	41.20	67.00	90.94

* Average wilting coefficient to depth of 3 feet, 7.1%.

wheatgrass, grama grass, and needle-and-thread in the uplands and sagebrush types. Thus there would seem to be a relation between high values for free water, high values for grams of bound water, and high values for total moisture content

SANDGRASS TYPE

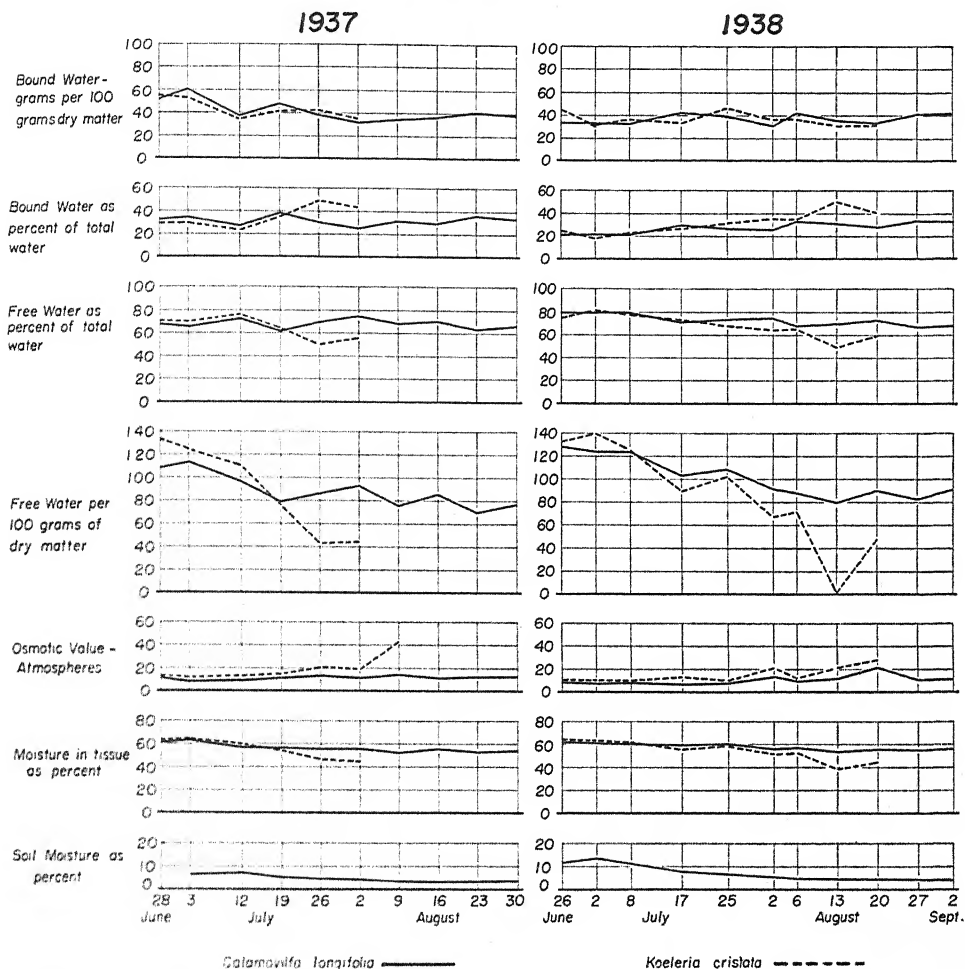


FIG. 3.—Changes in bound and free water, osmotic value, and total moisture in leaf tissues of sandgrass and Junegrass in sandgrass type during summer seasons of 1937 and 1938. Soil moisture also given.

of the tissue. On the other hand, high values for percentage bound water seem to be related to relatively low values for free water and total moisture content of the tissue.

4. BIG BLUESTEM TYPE.—The behavior of the species in this type, as illustrated in figure 4, is typically that to be expected when no stress is placed upon the plants by scarcity of available soil moisture. Although the calculated wilting coefficient

BIG BLUESTEM TYPE

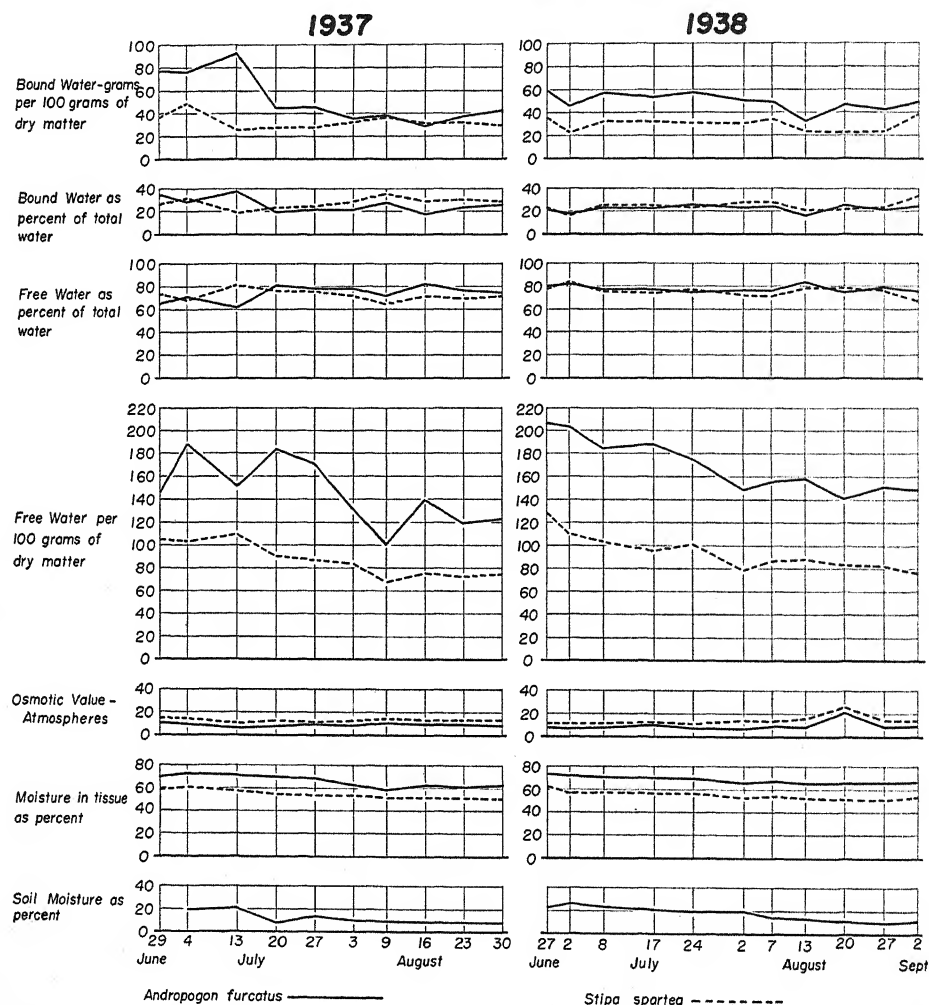


FIG. 4.—Changes in bound and free water, osmotic value, and total moisture in leaf tissues of big bluestem and porcupine grass in big bluestem type during summer seasons of 1937 and 1938. Soil moisture also included.

for the soil in this area is 10 per cent, the soil moisture remained above this point until the first part of August in 1937, and in 1938 it did not fall below the level of the wilting coefficient during the entire sampling period.

TABLE 8

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN BIG BLUESTEM TYPE, 1937 SEASON

DATE	SOIL MOIS- TURE* (%)	ANDROPOGON FURCATUS						STIPA SPARTEA					
		MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O /100 GRAMS /100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O /100 GRAMS /100 GM. DRY MATTER	MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O /100 GRAMS /100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O /100 GRAMS /100 GM. DRY MATTER
6/29.....	69.0	10.30	34.62	77.12	65.38	145.54	58.6	14.81	26.07	36.97	73.93	104.82
7/4.....	19.3	72.6	9.38	28.75	76.10	71.25	188.57	60.3	14.16	31.95	48.55	68.95	103.39
7/13.....	20.3	71.0	6.16	37.94	92.79	62.06	151.75	57.4	10.85	18.74	25.23	81.26	109.39
7/20.....	8.5	60.5	7.40	19.64	44.75	80.36	183.06	54.2	12.45	23.39	27.67	76.61	90.61
7/27.....	14.2	68.3	9.49	21.15	45.67	78.85	170.22	53.5	11.69	24.32	27.96	75.68	86.99
8/3.....	10.6	62.5	8.92	21.14	35.26	78.86	131.56	53.7	12.04	28.25	32.82	71.75	83.34
8/9.....	9.3	58.2	10.77	27.46	38.26	72.54	101.06	51.1	14.50	35.32	36.98	64.68	67.71
8/16.....	8.5	62.8	9.73	17.41	29.34	82.59	139.19	51.3	13.38	28.58	30.07	71.42	75.15
8/23.....	8.8	60.9	9.95	23.42	30.48	76.58	119.31	51.1	13.70	30.66	31.98	69.34	72.33
8/30.....	8.3	62.2	8.96	25.42	41.84	74.58	122.79	50.8	13.72	28.21	29.16	71.79	74.23

* Average wilting coefficient to depth of 36 inches, 10.0%.

TABLE 9

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN BIG BLUESTEM TYPE, 1938 SEASON

DATE	SOIL MOIS- TURE* (%)	ANDROPOGON FURCATUS						STIPA SPARTEA					
		MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER	MOIS- TURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H ₂ O % TOTAL H ₂ O	BOUND H ₂ O GRAMS/100 GM. DRY MATTER	FREE H ₂ O % TOTAL H ₂ O	FREE H ₂ O GRAMS/100 GM. DRY MATTER
6/27....	22.8	73.3	7.76	21.76	59.17	78.24	216.41	62.1	11.16	21.88	35.94	78.12	128.19
7/2.....	26.5	71.4	6.97	17.43	45.10	82.57	213.68	57.0	11.22	16.98	22.50	83.02	110.20
7/8.....	23.1	70.8	7.61	23.85	57.37	76.15	184.22	57.6	11.81	24.21	32.90	75.79	103.06
7/17....	20.3	70.7	9.77	22.21	53.41	77.79	188.20	56.2	12.50	25.42	32.72	74.58	95.95
7/24....	19.7	70.0	7.07	24.65	57.47	75.35	175.85	56.9	11.40	23.46	30.95	76.54	101.04
8/2.....	19.1	65.8	6.57	23.35	45.25	76.65	148.48	52.9	14.48	28.00	30.82	72.00	78.98
8/7.....	14.4	67.1	9.09	24.18	40.55	75.82	155.17	54.8	13.06	28.68	34.84	71.32	86.74
8/13....	13.3	65.6	8.83	16.81	32.97	83.19	158.33	52.9	16.20	21.13	23.82	78.87	88.40
8/20....	11.4	65.1	21.63	25.03	40.96	74.97	140.12	51.6	20.19	21.61	23.04	78.39	83.52
8/27....	10.0	66.0	8.84	21.93	42.74	78.07	151.72	51.3	14.36	22.39	23.60	77.70	82.04
9/2.....	11.1	66.4	9.48	24.96	49.31	75.04	148.22	53.5	14.68	33.26	38.50	66.74	76.99

* Average wilting coefficient to depth of 36 inches, 10.0%.

The data do not indicate any appreciable increase or decrease in percentage bound water and percentage free water. The osmotic values during the sampling period in 1937 remained on practically the same level, while in 1938 they showed only slight increases. The moisture percentage in the tissue of both species showed small but consistent decreases, and the decrease in grams of free water per 100 gm. of dry matter is large enough in both species to possess considerable significance. Grams of bound water per 100 gm. of dry matter show a fairly narrow range, with the exception of the values obtained for big bluestem in the early part of the 1937 season.

The entire behavior pattern of these species, as contrasted with the patterns obtained in the uplands and sagebrush types (figs. 1, 2), indicates a slow loss of moisture from the plant tissue with increasing maturity, rather than a relatively abrupt loss of moisture occasioned by the moisture level in the soil being reduced below the wilting coefficient. There is considerable similarity between the behavior of the species in the big bluestem type and in the sandgrass type. Sandgrass especially seemed to behave much as did big bluestem and porcupine grass.

Porcupine grass was considerably lower throughout the season in percentage moisture in the tissue and in grams of free water per 100 gm. of dry matter than was big bluestem (fig. 4). On the other hand, it was higher in osmotic value and averaged higher in percentage bound water for both seasons than did big bluestem (table 3). Big bluestem, with a much higher total moisture level and much greater values for grams of free water per 100 gm. of dry matter, showed higher values for grams of bound water per 100 gm. of dry matter than did porcupine grass. Not only this, but the average values of grams of bound water per 100 gm. of dry matter in big bluestem were higher than for any other species in any of the types, indicating again that the absolute amounts of bound water are closely related to the total moisture content of the tissue.

The average values for the various factors for both species in this type (table 3) show some significant differences when contrasted with the average values for the species in the other types. The average osmotic value for both species in the big bluestem type was considerably lower than the averages in any of the other types. The average for bound water percentage was also lower. On the other hand, the average bound water content of the two species expressed in terms of grams per 100 gm. of dry matter was approximately on the same level in the 1937 season as it was for the species in the other types. In the 1938 season the average of grams of bound water for the species in the big bluestem type was appreciably higher than the averages of this value for the species in the other types. As previously mentioned, the reason for this lies in the unusually high values for this factor shown by big bluestem. Another outstanding difference between the types is shown by the

much higher values for grams of free water recorded for the species in the big bluestem type. This again is the result of the remarkably higher average values of grams of free water in big bluestem.

Discussion

The results of this study seem to indicate that the bound water expressed as percentage of total water in the tissue, the percentage of total water in the tissue, the percentage of free water, and the grams of free water per 100 gm. of dry matter give the best expression of the responses of the plant species to the conditions of their habitats.

In the uplands type, where soil moisture is reduced below the wilting coefficient relatively early and the plants are placed under extreme stress for water, the species showed marked increases in bound water as percentage of total water and marked decreases in percentage of free water and in grams of free water per 100 gm. of dry matter.

In the sagebrush type, where conditions are similar to those in the uplands type, the species also showed marked increases in bound water as percentage of total water and marked decreases in percentage free water, and in grams of free water per 100 gm. of dry matter, as the season progressed. Both types represent relatively xerophytic habitats.

In the sandgrass type, which is not so xerophytic as the uplands and sagebrush types, only slight increases in percentage bound water occurred in the leaf tissue of the species during the course of the sampling period. Sandgrass showed less change than did Junegrass in percentage bound water and in percentage free water. Fairly large decreases in grams of free water per 100 gm. of dry matter were observed in both species, with larger decreases shown by Junegrass than by sandgrass.

In the big bluestem type, which is more mesophytic than any of the other three types, and where soil moisture was adequate throughout the period of sampling, increases in percentage bound water were relatively small, as were decreases in percentage free water and in grams of free water per 100 gm. of dry matter in the leaf tissue of the species.

The bound water expressed as grams per 100 gm. of dry matter did not seem to give an easily interpretable expression of the response of the species to the conditions of the habitat. Rather, this value seemed to be a characteristic of the individual species, which may or may not have been related to its ability to endure xerophytic conditions. In practically all species in all the types, the absolute amount of bound water (that is, bound water expressed on a dry-weight basis) tended to remain on somewhat the same level throughout the sampling period. This was illustrated especially in the species in the uplands and sagebrush types. Despite the large increases in percentage bound water, the values of grams of

bound water per 100 gm. of dry matter in the species in these two types in most cases showed only relatively small increases or decreases during the sampling period. In the sandgrass and big bluestem types the same general behavior was exhibited by the species, except that it was not so striking because the range of change in the other factors was so much smaller.

With the absolute amounts of bound water in the leaf tissue of the different species remaining on somewhat the same level, it is obvious that any losses in free water will be reflected as increases in percentage bound water. Thus the increase in percentage bound water in the species as the season progresses is primarily an accompaniment to the drying of the tissue. The actual amount of water held in the bound state in the plant tissue may even be decreasing under these conditions.

In habitats where soil moisture is inadequate, therefore, the tissues of the plants will be subjected to greater drying influences and the species will have a lower total water content, less free water per gram of dry matter, and consequently, higher percentages of bound water. At the beginning of the sampling periods, when soil moisture was adequate in all types and the percentage of moisture in the tissue was at a relatively high level in all species, values of percentage bound water were relatively low and approximately on the same general level for all species in all four types.

With the progressive seasonal reduction in soil moisture in the types, the leaf tissues of the plants began to lose moisture. These losses in total water in the tissue and in free water are reflected as increases in the bound water as percentage of the total water. The percentage of bound water in a given species is then a reflection of the extent to which drying of the tissue has taken place. Since this in turn is related to soil moisture, there seems to be a somewhat close relationship between soil moisture and percentage bound water. Consequently, the bound water percentage is primarily a reflection of the relative degree of dryness of the habitat, and not a measure of the adaptation of that species to a dry habitat.

This does not obviate the fact that the absolute bound water content of a species may be related to its ability to withstand drought conditions. It does indicate that this relationship is complex and the nature of it may not be revealed by gross measurements of the bound water content of the leaf tissues of a species growing in a given habitat.

Summary

1. A study of the bound and free water content of several prairie grasses in four representative grassland types in western North Dakota was made during the summer seasons of 1937 and 1938. The principal grass species in each of the four types were sampled once each week during the season for determinations of total water content, osmotic value of expressed sap, and relative proportions of bound and free water.

2. The bound water percentage, percentage free water, and grams of free water per 100 gm. of dry matter seem to give the best expression of the responses of the species to the conditions of their habitats.

3. Increases in bound water as percentage of total water resulted from losses in free water caused by progressive drying of the plant tissues and not from increases in the water-retaining capacity of the leaves. The bound water percentage is thus a reflection of the relative degree of dryness of the habitat and not a measure of the inherent adaptation of that species to a dry habitat.

4. The absolute amount of bound water present in the plant tissues, expressed as grams of bound water per 100 gm. of dry matter, did not seem to give an easily interpretable expression of the responses of the species to habitat conditions. This value seemed to be characteristic of the individual species and in each remained on somewhat the same level during the sampling period. There were specific differences in absolute amounts of bound water between species, but these differences apparently were not a direct measure of the ability of the species to tolerate xerophytic conditions.

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LITERATURE CITED

1. BARINOVA, R. A., Dynamics of the carbohydrate-colloidal complex as a factor in the ability of sugar beets to resist drought. *Bull. Acad. Sci. U.S.S.R. Ser. Biol.* 1937:254-270. 1937. (*Chem. Abstr.* 33:6389. 1939.)
2. CALVERT, J., Diurnal variation in "bound" and "free" water and other factors in the sap expressed from the leaves of *Phalaris tuberosa*. *Protoplasma* 24:525-530. 1935.
3. ———, Drought resistance in wheat. The "bound" and "free" water of expressed sap from wheat leaves in relation to time and soil moisture. *Protoplasma* 24:505-524. 1935.
4. CHRYSLER, H. L., Amounts of bound and free water in an organic colloid at different degrees of hydration. *Plant Physiol.* 9:143-155. 1934.
5. GREATHOUSE, G. A., Unfreezable and freezable water equilibrium in plant tissues as influenced by sub-zero temperatures. *Plant Physiol.* 10:781-788. 1935.
6. HANSON, H. C., and WHITMAN, W. C., Characteristics of major grassland types in western North Dakota. *Ecol. Monogr.* 8:57-114. 1938.

7. LEBEDINCEV, E., A study of the water-retaining capacity in relation to drought and frost resistance. Bull. Appl. Bot. Gen. and Plant Breed. 23:1-30. 1930.
8. MAXIMOV, N. A., Internal factors of frost and drought resistance in plants. Protoplasma 7:259-291. 1929.
9. MALLERY, T. D., Changes in the osmotic value of the expressed sap of leaves and small twigs of *Larrea tridentata* as influenced by environmental conditions. Ecol. Monogr. 5:1-35. 1935.
10. ———, Comparison of the heating and freezing methods of killing plant material for cryoscopic determinations. Plant Physiol. 9:369-375. 1934.
11. MEYER, B. S., Further studies on cold resistance in evergreens, with special reference to the possible role of bound water. BOT. GAZ. 94:297-321. 1932.
12. MÜLLER-THURGAU, H., Ueber das Gefrieren und Erfrieren der Pflanzen. Landw. Jahrb. 9:133-189. 1880.
13. NEWTON, R. J., and GORTNER, R. A., A method for estimating hydrophilic colloid content of expressed plant tissue fluids. BOT. GAZ. 74:442-446. 1922.
14. NEWTON, R. J., and MARTIN, W. M., Physico-chemical studies on the nature of drought resistance in crop plants. Canad. Jour. Res. 3:336-427. 1930.
15. NOVIKOV, V. A., Investigations on the drought resistance of plants. Jour. Agr. Sci. of S.E.-U.S.S.R. 9:47-72. 1931.
16. ROBINSON, W., Free and bound water determinations by the heat of fusion of ice method. Jour. Biol. Chem. 92:699-709. 1931.
17. RÜBNER, M., Über der Wasserbindung in Kolloiden mit besonderer Berücksichtigung des quergestreiften muskels. Abh. preuss. Akad. Wiss. Phys.-Math. Klassen. 1922:3-70. 1922.
18. SAYRE, J. D., Methods of determining bound water in plant tissue. Jour. Agr. Res. 44:669-688. 1932.
19. SCHOPMEYER, C. S., Transpiration and physico-chemical properties of leaves as related to drought resistance in loblolly pine and shortleaf pine. Plant Physiol. 14:447-462. 1939.
20. SPOEHR, H. A., The carbohydrate economy of cacti. Carnegie Inst. Wash. Publ. 287. 1919.
21. STARK, A. L., An apparatus and method for determining bound water in plant tissue. Proc. Amer. Soc. Hort. Sci. 29:384-388. 1932.
22. ———, Unfrozen water in apple shoots as related to their winter hardiness. Plant Physiol. 11:689-711. 1936.
23. ST. JOHN, J. L., The temperature at which unbound water is completely frozen in a biocolloid. Jour. Amer. Chem. Soc. 53:4014-4019. 1931.
24. THOENES, F., Untersuchungen zur Frage der Wasserbindung in Kolloiden und tierschen Geweben. Biochem. Zeitschr. 157:174-186. 1925.
25. VASSILIEV, I. M., and VASSILIEV, M. G., Changes in carbohydrate content of wheat plants during the process of hardening for drought resistance. Plant Physiol. 11:115-125. 1936.
26. WALTER, H., Neue Gesichtspunkte zur Beurteilung der Wasserökologie der Pflanzen. Ber. Deutsch. Bot. Ges. 47:243-252. 1928.
27. WELCH, W. B., Water relations in *Bryophyllum calycinum* subjected to severe drying. Plant Physiol. 13:469-487. 1938.

CHEMICALLY INDUCED PARTHENO-CARPY IN CERTAIN HORTICULTURAL PLANTS, WITH SPECIAL REFERENCE TO THE WATERMELON¹

C. Y. WONG

(WITH EIGHT FIGURES)

Introduction

Natural parthenocarp is of common occurrence in banana, citrus, vinifera grape, Chinese persimmon, and English forcing cucumber. Induced or stimulative parthenocarp in many species and varieties has been observed to occur as a result of certain internal and external influences. Since parthenocarpic fruits are considered desirable, efforts have been made: (a) to obtain seedless fruits by breeding or selection, (b) to induce parthenocarpic in place of non-parthenocarpic fruits, and (c) to increase the percentage of parthenocarpic fruits where some occur normally.

The object of this study was to determine the possibility of inducing parthenocarp by the use of synthetic growth substances in some horticultural crops, especially the watermelon. More specifically it aimed to determine: (1) the response of certain horticultural plants to parthenocarpic and apomictic phenomena, (2) the kinds and concentrations of growth substances that give best results, (3) the fruit and seed development in parthenocarpic as compared with normal seed-bearing fruit, and (4) the possibility for practical application of the results obtained.

Review of literature

As early as 1902, MASSART (11) placed dead pollen upon the stigma of an orchid and observed a slight growth of the ovary. HARTLEY (10) obtained parthenocarpic fruit of tobacco by the use of *Azalea* pollen. FITTING (2, 3), using dead and living pollen and pollen extracts, was able to cause some slight growth in the ovary of several species of orchids. WINGE (15) found that large fruits with sterile seeds could be produced in hops pollinated with hemp or *Urtica* pollen.

In attempts to produce parthenocarp by cross pollination and other means, only some of which were successful, YASUDA (16, 17, 18, 20) obtained parthenocarpic eggplant, tomato, and pepper fruits by pollination with pollen other than their own. He also obtained satisfactory results by injecting aqueous extracts of pollen into the ovary. In cucumber and a solanaceous plant, however, partheno-

¹ Journal Article no. 476 (N.S.) from the Michigan Agricultural Experiment Station.

carpic development did not take place in flowers that opened early in the season, although it did appear at a later date.

In cases where parthenocarpic fruit developed, the pollen tubes grew into the style but did not reach the ovary. In later studies (19) the flowers of eggplant and cucumber were emasculated and pollinated with their own well-developed pollen grains. The pollinated stigmas and styles were then cut off at different periods following pollination. When the operation was delayed (24 hours in eggplant and 9 hours in cucumber), fertilization occurred and the ovaries developed into normal seeded fruits. If the operation was so timed that the majority of the pollen tubes reached the base of the style, some seedless fruits resulted. It was concluded that the pollen grains or pollen tubes produced some chemical substances which caused parthenocarpic development of the ovaries. Parthenocarpy, phenospermy (steno-spermy), and parthenogenesis were believed to be caused by the action of the same stimulating substance. It was further concluded that parthenogenesis would take place when the pollen-tube substance reached the ovules and stimulated them without fertilization. On the other hand, when the substance arrived at the base of the style or at the ovaries and stimulated the ovarian tissue alone, parthenocarpy would occur. Phenospermy may take place in an intermediate situation. By use of pollen extracts, GUSTAFSON (7) produced parthenocarpy in eggplant, pepper, cucumber, and certain ornamental plants.

Parthenocarpy induced by growth substances was first accomplished by GUSTAFSON (6) in 1936 by treating the pistils with known hormones in lanolin paste. He obtained normal-sized parthenocarpic fruits in tomato, *Petunia*, and *Salpiglossis* when the pistils were treated with indole-3n-a-propionic acid. Similar treatment initiated growth in snapdragon ovaries but did not produce mature fruits. Phenylacetic acid caused seedless tomato fruits to develop and slight growth in snapdragons, but none in tobacco. Indoleacetic acid resulted in seedless fruits in the tomato, *Salpiglossis*, *Petunia*, *Begonia*, pepper, and eggplant. The same substance initiated ovarian growth in the snapdragon, *Zephyranthes carinata*, *Agapanthus umbellatus*, Crookneck Summer squash, and Hubbard squash, but not in cucumber and watermelon. Indolebutyric acid induced seedless fruits in tomato, *Salpiglossis*, *Petunia*, Crookneck Summer squash, *Begonia*, pepper, and eggplant but produced only slight growth in the ovaries of Hubbard squash, cucumber, and watermelon.

As a result of his own work and that of other investigators, GUSTAFSON (8) suggested that some fruits develop without fertilization because they have a higher auxin content in the ovary at the time of blossoming. He believed this content high enough to initiate growth processes, with the result that the ovaries commence to develop, even though there has been no fertilization. He found that the auxin content of ovules and developing seeds is much greater than that of other

parts of the fruit; moreover, the auxin content, in pepper at least, was much lower in the winter and spring than in the summer.

HAGEMANN (9) obtained parthenocarpic gladiolus by means of indoleacetic acid, but negative results from phenylacetic acid. GARDNER and MARTH (4) induced parthenocarp in holly by spraying the open blossoms with naphthaleneacetic, indolebutyric, indoleacetic, and indolepropionic acids. The effectiveness of the substances followed the descending order as listed. They also found that better results were obtained by repeated applications. Moreover, parthenocarpic fruits were produced by watering the soil during full bloom with indoleacetic acid. They also obtained some parthenocarpic fruit by introducing small quantities of indoleacetic acid in powder form into holes in the stem made with a small nail. In a pistillate strawberry, parthenocarpic fruits of normal size but with empty achenes were induced by these workers, using the substances just mentioned in spray form. Negative results were obtained in the Starking apple from indoleacetic-acid spray and in the Brighton grape from naphthaleneacetic-acid treatment. In a further study, GARDNER and MARTH (5) found that naphthalene acetamide gave better results than naphthaleneacetic acid in inducing parthenocarp in holly. Potassium-naphthalene acetate gave results far inferior to those obtained from phenylacetic acid and sodium-naphthol sulphonate. In studying the effect of certain growth substances on inflorescences of dates, NIXON and GARDNER (12) showed that 1 per cent naphthaleneacetic acid in lanolin paste applied to Thoory date arrested senescence of the perianth and strand. Negative results were obtained from indoleacetic and indolebutyric acids. In the Deglet Noor variety, spraying with naphthaleneacetic acid in aqueous solution about 10 days after pollination reduced the set by about 50 per cent.

Material and methods

This study was conducted at Michigan State College, East Lansing, during the summer of 1938 and the spring and summer of 1939. The varieties used were:

WATERMELON (*Citrullus vulgaris*): Winter Sweet, Fordhook Early, Early Arizona, Favorite Honey, Harris Earliest, Best Early, Select Early, Sweet Japanese, Tough Sweet, Northern Sweet (Selection nos. 1, 2, and 5), Coles Early, Early Kansas, Stone Mountain no. 5, Stone Mountain, Kleckley Sweet no. 6, Hawkbury, Iowa 1, Iowa 3, Iowa 5, "Yellow Melon," and six selected strains.

CUCUMBER (*Cucumis sativus*): National Pickling.

MUSKMELON (*Cucumis melo* var. *reticulatus*): An unnamed selection for the greenhouse tests in the spring 1939 and the variety Honey Rock for the summer experiments.

PUMPKINS AND SQUASHES (*Cucurbita pepo*): Early Prolific Straightneck, Dark Green Zucchini, Omaha, Delicata, Fort Berthol, White Bush Scallop, Table Queen, and Hardin Bush.

SQUASHES (*Curcubita maxima*): Buttercup.

SQUASH (*Cucurbita moschata*): African Bell.

In addition, certain cucurbits of uncertain species carrying U.S.D.A. Plant Introduction nos. 127585, 127586, 127588, 127589, and 127590.

PEPPER (*Capsicum frutescens*): Harris Wonder.

TOMATO (*Lycopersicum esculentum*): Michigan State Forcing.

EGGPLANT (*Solanum melongena*): New Hampshire Hybrid.

STRAWBERRY (*Fragaria*): An unnamed variety of the everbearing type.

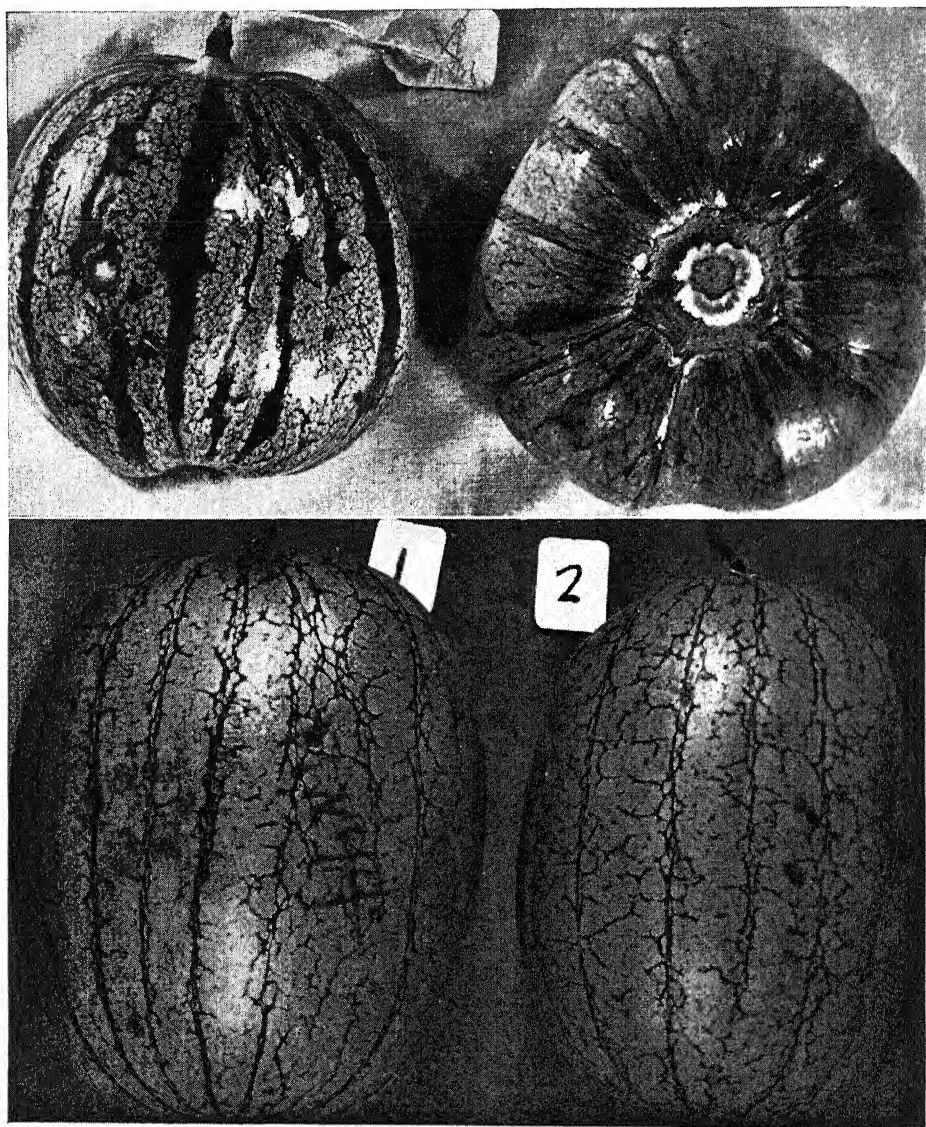
The Winter Sweet watermelon (fig. 1), also known as Dakota Sweet, is of medium size, red fleshed, weighing 9-14 pounds, with about 600 seeds per fruit. It is andromonoecious with occasional monoecious flowers. Northern Sweet is closely related to Winter Sweet, differing little either in external or internal characteristics. Favorite Honey (fig. 2) is a small, early, and very prolific melon with yellow flesh. It weighs 3-4 pounds and has about 250 seeds per fruit. The strains designated as Selection nos. 1-6 were derived from crosses between Favorite Honey and Winter Sweet. The seed of the Yellow Melon was purchased from a commercial firm as Favorite Honey, but later it was found to differ from Favorite Honey from other sources. This melon was much larger in size, averaging about 8-12 pounds, with very light lemon-yellow flesh. Its rind is exceptionally crisp and thin so that the fruits crack open easily. There are about 900 seeds per fruit, and the variety is andromonoecious. The sex condition of the other watermelon varieties was mostly monoecious, although occasionally hermaphrodite flowers were observed.

The synthetic growth substances used were naphthaleneacetic acid, potassium-naphthalene acetate, indolebutyric acid, colchicine, acenaphthene, sulfanilamide, and trimethylamine, mostly in lanolin paste. Naphthaleneacetic acid was also used as a spray by means of a hand atomizer.

The chemicals were thoroughly mixed with lanolin and these preparations smeared either on the stigma (if the style was short) or on the cut surface of the style (if the latter was long). If the style was more than 2 mm. it was found desirable to cut it off about 1 mm. above the ovary and smear the lanolin preparation on this surface.² Pastes prepared from growth substances were used in concentrations of 1, 2, 2.5, and 5 per cent. For spraying, 50 and 500 p.p.m. were used in the case of naphthaleneacetic acid and 40 p.p.m. in the case of trimethylamine. Since naphthaleneacetic acid is not soluble in water, the desired amount was first dissolved in a small quantity of alcohol, which was then added to the required amount of water.

The watermelons, cucumbers, muskmelons, eggplants, and peppers used in the field experiments were started in the greenhouse and transplanted to the field later. Some of the watermelon seeds were pre-treated with different chemicals, such as

² GUSTAFSON, F. G., Communication of March 5, 1937.



FIGS. 1, 2.—Fig. 1 (above), Winter Sweet watermelon: side and blossom-end view of parthenocarpic fruit produced by treating style with naphthaleneacetic acid in lanolin paste before anthesis. Note marked protruding styler scar tissue in blossom-end portion. Fig. 2 (below), Favorite Honey watermelon: (1) from self-pollination with about 20 grains of pollen and with K-naphthalene acetate paste added; (2) from naturally pollinated pistil.

colchicine, acenaphthene, ether, and water, for various lengths of time before sowing.

Usually the flowers of each variety were subjected to four kinds of treatments: (1) pollinated in the usual way, (2) self-pollinated by hand, (3) treated with the substances under investigation, and (4) neither pollinated nor treated.

Flower buds nearly ready to open—but before anthesis had taken place—were selected. In hermaphroditic flowers, emasculation was carried out the day before treatment and the flowers protected with a wire cage. In a later experiment the style was cut off just above the ovary with a sharp scalpel, and the cut surface either smeared with the lanolin preparation or left without treatment as a control. Flowers so treated were not caged. A careful trial by GUSTAFSON (6), further confirmed by the writer, showed that if pollen is placed on the cut surface of the style there is never any development of the ovary to indicate that fertilization had taken place.

Investigation

EXPERIMENTS WITH WATERMELON

PRELIMINARY TRIALS (1938).—These included nine varieties and thirteen treatments. Pistils were not treated until after each plant had developed at least one rather good-sized fruit from self-pollination. Apparently this pollinated, seed-bearing fruit exerted some inhibiting effect on, or led to an unfavorable condition for, parthenocarpic development, thus explaining the poor results obtained (table 1).

No fruits were formed where emasculated flowers were left unpollinated or where the styles were cut and then lanolin paste, pollen, or indolebutyric acid applied to the cut surface. On the other hand, fruit development was induced by application of naphthaleneacetic acid, either to the stigmas or to the cut style (fig. 1). These watermelons were seedless and also lacked seed coats, with the exception of those of the variety Harris Earliest. They varied considerably in shape and size. In general they were slightly angular or even ribbed, although some were normal in shape and size. They were solid and firm in texture, and the flesh showed an intense red coloration. No difference in flavor could be detected when compared with normally pollinated fruits.

The three plants of the Winter Sweet watermelon grown from seed which had first been subjected to colchicine treatment showed a typical colchicine effect—stunting early in the season, large leaves and flowers, and great vigor later in the season. Although pollen was present in considerable abundance, it failed to induce fruit setting when the blossoms were selfed; on the other hand, hormone-treated flowers set satisfactorily.

In a parallel experiment involving colchicine pre-treated plants, a small fruit

was produced by self-pollination and a large one by treatment with 1 per cent naphthaleneacetic acid in lanolin paste. Both flowers were treated at the same time. The fruit from selfing dropped within 10 days, but the hormone-treated fruit continued to grow until maturity. Nevertheless, fruits containing apparently normal seeds were formed from some open-pollinated flowers (fig. 3*B*). Probably this was due to fertilization by nearby noncolchicine-treated plants. It is a case of vicinism. These fruits were much smaller in size and slower in growth than those from the hormone-treated blossoms.

TABLE 1

WATERMELONS: FRUIT SETTING AS RESULT OF HORMONE TREATMENT; 1938
DATA FOR NINE VARIETIES GROUPED TOGETHER

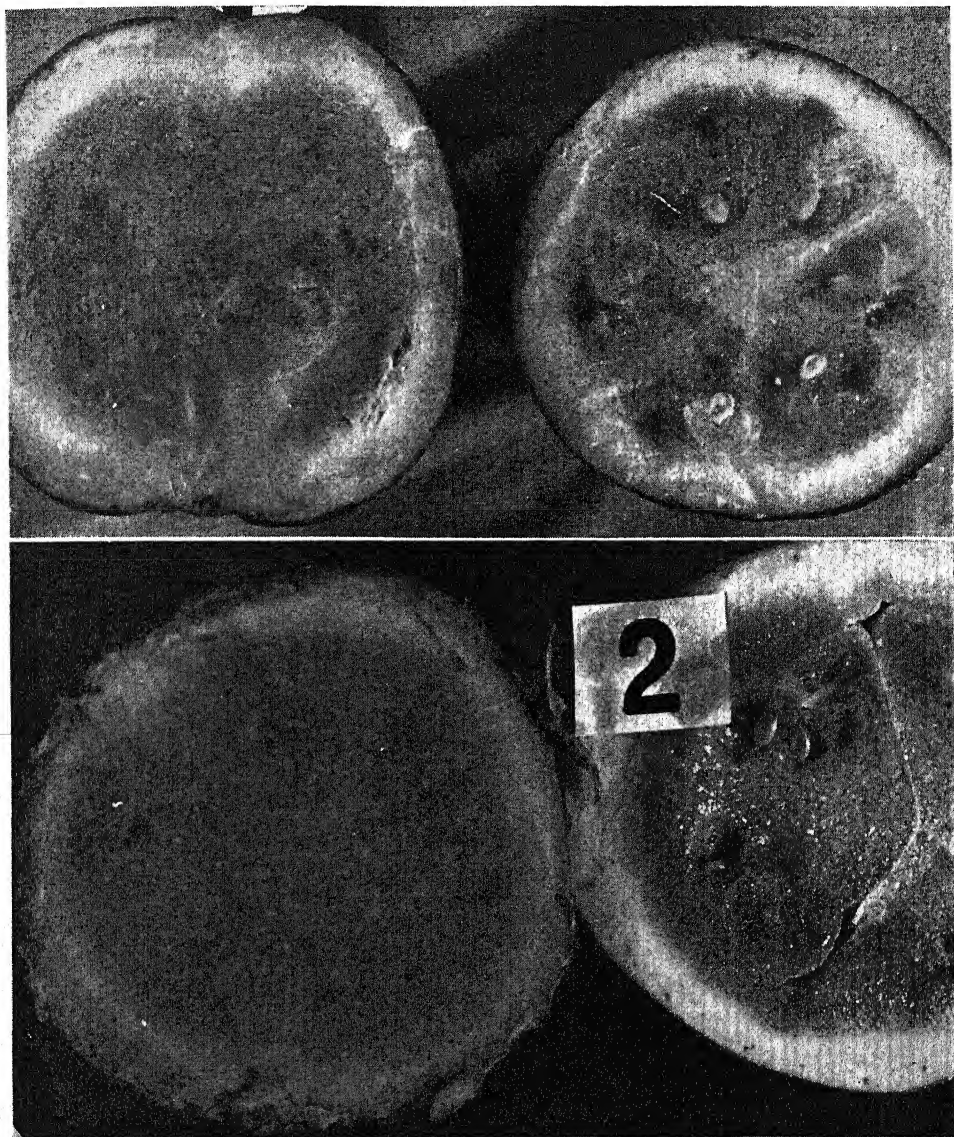
TREATMENT	No. OF BLOS- SOMS	No. SET	PER- CENT- AGE SET	REMARKS
2.5% iba* in lanolin paste applied to cut style.....	42	0	0
5% iba in lanolin paste applied to cut style.....	17	0	0
2.5% iba paste applied to stigma.....	11	0	0
Check, no treatment except emasculation..	15	0	0
Cut style only.....	7	0	0
Pollen applied to cut style.....	7	0	0
Lanolin paste applied to cut style.....	10	0	0
2.5% naa paste applied to cut styles.....	10	8	80	Seven stopped grow- ing at about 1½ in. diameter
5% naa paste applied to cut style†.....	15	3	20	Fruits small and ir- regular shaped
2.5% naa paste applied to cut style†.....	12	4	33
1% naa paste applied to cut style†.....	11	4	36
Self-pollinated†.....	10	0	0
Stigma sprayed with 0.05% naa solution†.	2	2	100	Treated very late in season

* These abbreviations are used in this and the following tables: naa = naphthaleneacetic acid; kna = potassium-naphthalene acetate; iba = indolebutyric acid; a = acenaphthene; s = sulfanilamide; c = colchicine.

† Seeds pre-soaked in 0.4% colchicine aqueous solution for 4 days at room temperature.

GREENHOUSE TRIALS (1939).—About two dozen flower buds from Selection nos. 1, 2, and 5 were treated. Acenaphthene powder was first used to cover the cut styles, which were then covered with a lanolin paste containing 1 per cent naphthaleneacetic acid. Four fruits of normal size were ripened, 1900–3241 gm. in weight. They contained hard seed coats of about normal size but without embryos. It is a case of stenospermy. Three small fruits entirely lacking in edible flesh were also produced.

FIELD EXPERIMENTS (1939).—These included twenty-one varieties with sixteen treatments. The results are presented in table 2. Ovaries of flowers with treated stigmas or cut styles showed various responses. Some failed to develop at all;



FIGS. 3, 4.—Fig. 3 (above), Winter Sweet watermelon: left, longisecton of naphthaleneacetic acid-treated fruit; right, cross section of open pollinated fruit. Note absence of seed coats in *A*. Seed from which plant was grown was soaked in 0.4 per cent colchicine solution for 4 days. Fig. 4 (below), Winter Sweet watermelon: two watermelons produced from naphthaleneacetic-acid treatment. The one without seeds and seed coats grew on a vine produced from seed pre-treated with colchicine; the fruit on the right produced what appeared to be seeds, but which were in reality undersized seed coats. Seed from which this plant was grown was given no such pre-treatment. Note greatly thickened rind in both cases.

others developed into small fruits which remained on the vine but never grew normally; others developed into normal fruits, except for the absence of seed.

Table 2 shows that a mixture of two growth substances gave better results than one alone. For example, when acenaphthene and naphthaleneacetic acid were used together, the percentage of parthenocarpic fruits produced was 7.2, as compared with 2.3 and 0 when naphthaleneacetic acid and acenaphthene, respectively, were

TABLE 2

WATERMELONS: FRUIT AND CROP SETTING AS RESULT OF APPLICATIONS OF HORMONES IN LANOLIN PASTE TO CUT STYLE; 1939

TREATMENT	No. OF FLOWERS TREATED	FRUIT SET*		CROP SET*		No. OF VARIETIES USED
		No.	%	No.	%	
1% naa.....	322	69	21.4	14	2.3	10
2% naa.....	402	149	37.1	29	7.2	13
1% kna.....	306	62	20.3	5	1.6	11
2% kna.....	309	97	31.4	16	5.2	12
1% each of naa and iba.....	301	87	28.9	15	5.0	12
1% naa plus 10% a.....	430	143	33.3	31	7.2	10
1% kna plus 0.1% c.....	152	46	30.3	4	2.6	8
1% kna plus 0.5% c.....	106	46	43.4	15	14.2	7
1% kna, 1% a, 1% iba, and 0.1% c.....	101	39	38.6	9	8.9	6
About 20 grains of pollen applied to stigma	103	2	1.9	1	1.0	5
About 20 grains of pollen applied to stigma plus:						
1% kna paste to stigma.....	102	22	21.6	3	2.9	7
1.5% s.....	50	0	0	0
10% a.....	84	0	0	0
Check, cut style only.....	524	0	0	0	13
Selling of one-lobed stigma and removing of other two lobes.....	29	2	6.9	1	3.5	5
Self-pollinated.....	180	80	44.4	66	36.7

* The terms fruit set and crop set in this and following tables are used to describe the degree of development obtained: fruit set applies to fruits 1.5-12.5 cm. in diameter, while crop set applies to fruits more than 12.5 cm. in diameter.

used alone. Indolebutyric acid failed to induce parthenocarpic development at concentrations of 1, 2.5, and 5 per cent but when a mixture of 1 per cent each of indolebutyric and naphthaleneacetic acids was used, 5 per cent of parthenocarpic fruits of normal size were obtained.

In these experiments a concentration of 2 per cent of the different chemicals gave better results than 1 per cent. There was some indication that the naphthaleneacetic acid had a greater influence on parthenocarpic development than its potassium salt.

To determine whether a watermelon fruit can develop with a very limited number of seeds, about twenty grains of pollen were applied to the stigmas of each of a number of flowers. Only one fruit developed from the 103 flowers treated in this way. By adding a growth substance (K-naphthalene acetate) to the stigma, how-

ever, followed by application of a limited amount of pollen, three mature fruits were obtained from 102 treated flowers. In general, all the hormone-treated ovaries that did not develop into mature fruits enlarged to several times their original size, then ceased further growth, turned soft, and dried but persisted on the vine, even when the plants were killed by the first frost. On the contrary, the untreated and unpollinated ovaries were shed within 7-10 days.

TABLE 3
WATERMELONS: RESPONSE TO THE VARIOUS HORMONE
TREATMENTS LISTED IN TABLE 2*

VARIETY	No. OF FLOWERS TREATED	FRUIT SET		CROP SET	
		No.	%	No.	%
Winter Sweet.....	567	187	32.9	31	5.5
Northern Sweet.....	540	96	17.7	2	0.4
Favorite Honey.....	204	69	33.8	40	19.6
Coles Early.....	64	33	51.5	3	4.7
Early Kansas.....	45	10	22.2	1	2.2
Fordhook Early.....	94	30	31.9	2	2.1
Stone Mountain.....	62	26	41.9	2	3.2
Kleckley Sweet.....	34	15	44.1	0
Hawksbury.....	83	15	18.0	1	1.2
Iowa 5.....	26	10	38.4	1	3.8
Selection 1.....	45	0	0
Selection 2.....	11	5	45.4	5	45.5
Selection 3.....	2	0
Selection 4.....	11	2	18.1
Selection 5.....	58	10	17.2	4	6.9
Harris Earliest.....	10	2	20.0	0
Yellow Melon.....	565	226	40.0	46	8.1

* With the exception of pollination, check and treatments with acenaphthene and sulfanilamide.

Colchicine, when mixed with the synthetic growth-substance preparation, gave an increase in the percentage of parthenocarpic fruits from any treatment: a 14.2 per cent set resulted from a mixture of 1 per cent naphthaleneacetic acid and 0.5 per cent colchicine preparation. Probably colchicine may be considered as a growth substance from the standpoint of parthenocarpic development.

By removing two-thirds of the stigmatic surface and pollinating only the remaining portion, normal seed-bearing fruits were produced.

Differences in varietal response to various hormone treatments are presented in table 3.

Table 3 shows that varieties differ greatly in their ability to produce parthenocarpic fruit as a result of treatment. For instance, Favorite Honey and Yellow Melon gave high percentages of parthenocarpic fruits, but Early Kansas, Northern Sweet, and Selections 1, 3, and 4 produced none or very few. Northern Sweet is

probably closely related to Winter Sweet; however, the former variety gave a much lower percentage of parthenocarpy. Moreover, Selections 1-5 were all derived from a cross between Winter Sweet and Favorite Honey, but only Selections 3 and 5 produced parthenocarpic fruits.

Table 4 presents the responses of four representative varieties to the various hormone treatments. The responses agree closely with those shown in table 2.

TABLE 4
WATERMELONS: NUMBERS AND PERCENTAGES OF FRUIT AND CROP SETTING
WITH HORMONES IN LANOLIN PASTE APPLIED TO CUT STYLES

TREATMENT	WINTER SWEET			NORTHERN SWEET			FAVORITE HONEY			YELLOW MELON		
	A*	B*	C*	A	B	C	A	B	C	A	B	C
1% naa.....	85	24.7	2.4	95	5.3	0	25	16.0	12.0	70	40.0	7.1
2% naa.....	64	37.5	6.3	120	29.2	0	37	48.6	35.2	98	39.7	7.1
1% kna.....	39	20.5	2.6	66	6.1	0	11	27.3	18.2	102	29.4	1.0
2% kna.....	27	7.4	0	78	16.7	1.3	22	27.3	13.6	55	49.1	12.7
1% mixture of naa and iba.....	90	30.0	4.4	61	16.4	0	28	32.1	17.9	74	39.2	5.4
1% naa plus 10% a.	181	43.1	8.3	52	17.3	1.9	10	10.0	10.0	96	38.5	29.7
1% kna plus 0.1% c	26	50.0	3.8	43	23.3	0	22	22.7	4.5	15	60.0	13.3
1% kna plus 0.5% c	24	16.7	0	18	33.3	0	49	46.9	24.5	11	90.9	27.3
1% each of kna, a, iba, and 0.1% c.	31	32.3	3.2	7	57.1	0	44	38.6	13.6
About 20 grains of pollen applied to stigma.....	37	0	0	32	0	0	12	0	19	10.5	5.3
About 20 grains of pollen applied to stigma plus 1% kna paste to stig- ma.....	32	25.0	0	15	26.7	0	15	20.0	20.0	35	17.1	0
1.5% s.....	14	0	7	0	10	0	15	0
10% a.....	51	0	20	0
Check, cut style only.....	50	0	48	0	78	0	207	0
Self-pollination by hand.....	26	57.7	53.8	32	15.6	15.6	10	70.0	70.0	41	53.6	46.3

* A, number of flower treated; B, percentage of fruit setting; C, percentage of crop setting.

For instance, naphthaleneacetic acid gave a better set than its potassium salt, and a mixture gave more favorable results than one hormone alone. Favorite Honey gave the highest set with all treatments, but no initial development was noted when a very limited amount of pollen was applied. When pollen was added with a growth substance, however, fairly good results were obtained.

No initial growth was observed on the check flowers of any variety. If natural parthenocarpy could occur, it might be expected on Favorite Honey or Yellow Melon, which gave the highest percentage of stimulative parthenocarpy.

The varietal responses of watermelons to various treatments with respect to fruit size, shape index, and weight are presented in table 5. Although the means

TABLE 5

WATERMELONS: MEAN FRUIT SIZE, SHAPE INDEX, AND WEIGHT AS RESULT OF TREATMENT

VARIETY	TREATMENT	NO. OF FRUITS	DIAMETER (CM.)		SHAPE INDEX*	WEIGHT (GM.)
			POLAR	EQUATORIAL		
Winter Sweet...	1% naa	5	16.3	13.4	0.82	1551
	2% naa	4	16.3	14.3	0.88	1481
	1% kna	1	14.1	13.6	0.97	1205
	1% naa and 1% iba	4	17.2	14.0	0.80	1653
	1% naa, 10% a	14	15.9	13.7	0.86	1452
	1% naa, 0.1% c	1	21.0	16.9	0.80	2821
	1% each of kna, a, and iba; 0.1% c	1	25.1	15.1	0.60	2600
	1.5% s	6	19.9	19.5	0.98	3169
Northern Sweet.	2% kna	1	15.1	11.0	0.73	857
	1% naa, 10% a	1	23.1	16.6	0.72	3070
	1.5% s	8	22.4	20.9	0.93	4195
Favorite Honey...	1% naa	3	13.9	13.0	0.93	1133
	2% naa	13	14.7	12.1	0.82	1089
	1% kna	2	16.5	12.9	0.78	1455
	2% kna	3	13.8	11.1	0.81	983
	1% naa, 1% iba	5	19.0	14.0	0.74	1628
	1% naa, 10% a	1	15.0	12.0	0.80	1300
	1% naa, 0.1% c	1	12.6	10.3	0.82	733
	5% naa, 0.1% c	10	14.2	12.3	0.87	1158
	About 20 pollen grains; 1% kna	3	22.3	15.3	0.69	2374
	1.5% s	2	18.3	15.8	0.86	2062
Coles Early....	2% naa	1	18.9	15.1	0.80	2080
	2% naa, 10% a	2	23.0	18.0	0.78	3521
Early Kansas...	2% kna	1	17.5	12.0	0.69	920
Fordhook Early...	1% kna	1	21.4	19.2	0.90	3972
	1% naa, 10% a	1	19.2	13.8	0.72	1810
Stone Mountain.	1% each of kna, a, and iba; 0.1% c	2	14.9	12.3	0.83	533
Hawksbury.....	2% naa	1	23.2	12.8	0.55	1679
Iowa 5.....	2% kna	1	24.0	19.4	0.81	4008
Selection 2.....	2% naa	2	19.9	17.1	0.86	2379
	2% kna	2	15.6	15.6	1.00	1893
	1% naa, 10% a	1	13.8	12.1	0.88	894
	1.5% s	1	21.2	21.0	0.99	3850
Selection 5.....	1% naa	1	16.0	13.5	0.84
	2% naa	1	15.0	11.0	0.73	1008
	2% kna	1	14.6	12.6	0.86	1023
	1% naa, 10% a	1	13.8	11.8	0.86	1034
Yellow Melon...	1% naa	5	21.2	19.4	0.91	3603
	2% naa	6	18.4	16.5	0.90	2631
	1% kna	1	15.6	13.6	0.87	1439
	2% kna	7	21.4	20.0	0.94	3512
	1% naa, 1% iba	4	20.0	16.8	0.84	2535
	1% naa, 10% a	10	18.0	16.1	0.90	2617
	1% naa, 0.1% c	2	18.6	18.2	0.98	3142
	5% naa, 0.1% c	3	16.9	15.1	0.90	1985
	1% each of kna, a, and iba; 0.1% c	6	15.4	14.3	0.92	1701
	About 20 pollen grains	1	15.1	11.8	0.78	959
	Self pollinated	12	21.1	19.9	0.95	4048

* Shape Index = $\frac{\text{Equatorial diameter}}{\text{Polar diameter}}$.

represent only a limited number of fruit measurements, they indicate that the average size of the parthenocarpic fruit was less than that of pollinated seed-bearing fruits. However, parthenocarpic fruits of normal size or even larger than those from pollination were obtained in some varieties, such as Favorite Honey and Yellow Melon, when their ovaries were treated with certain growth substances. Moreover, many fruits of normal shape were produced as a result of parthenocarpic development, as indicated by the shape indices.

Most of the parthenocarpic fruits that developed to full size were normal in appearance, except that they lacked seeds. On the other hand, some fruits which can be induced to set parthenocarpically do not respond normally to treatment, as

TABLE 6
WATERMELONS: CHARACTERISTICS OF PARTHENOCARPCIC AND
POLLINATED SEED-BEARING FRUITS

VARIETY	TREAT- MENT	THICKNESS OF RIND (MM.)	COLOR OF FLESH*	HOLLOWNESS	STYLAR SCAR TISSUE
Winter Sweet...	P† H	9.5-4.7 15.9-8.6	Red (Vermilion 18/2) Deep red (Signal Red 719/2)	Hollow Solid or hollow	Sunken Protruding
Yellow Melon...	P H	9.2-4.3 12.8-7.0	Pale yellow (Aureolin 3/3) Orange yellow (Lemon Yellow 4/1 to Tangerine Yellow 9/3)	Hollow Mostly solid	Sunken Protruding

* Color description based on Horticultural Color Chart (1).

† P = pollination; H = hormone.

indicated by abnormal development of their various tissues. Their flesh (placentae) is lacking or only partially developed, or a light margin may occur in the place of contact between placental regions; there is often an increase in the thickness of rind, and intense coloration of flesh. The fruits may be slightly triangular or even ribbed. Comparisons between self-pollinated and parthenocarpic watermelons from all hormone treatments, with respect to rind thickness, flesh color, hollow-ness, and styler scar tissue, are presented in table 6.

The rind of the hormone-treated fruits was almost twice as thick as that of the pollinated ones. The coloration was more intense in the parthenocarpic fruits. The texture was firm and less juicy, especially in the Winter Sweet variety, when the seeds were pre-treated with colchicine (fig. 4). Another outstanding difference between them was their styler scar tissue, which protruded in the fruits which developed from applying the growth substance to the cut style (fig. 1).

The varietal responses with respect to the size and weight of seeds and seed coats as well as color and texture of seed coats from self-pollinated and parthenocarpic fruits in watermelons are presented in table 7 and figure 5. These data

show that no seed was developed in any of the treated fruits, although seed coats were developed to various degrees. Winter Sweet, Northern Sweet, Favorite

TABLE 7

WATERMELONS: VARIETAL RESPONSE IN SEED AND SEED-COAT DEVELOPMENT
AS SHOWN BY SELF-POLLINATED AND PARTHENO-CARPIC FRUITS

VARIETY	TREAT- MENT	SEED		SEED COAT		
		SIZE (MM.)	WEIGHT* (GM.)	SIZE (MM.)	WEIGHT (GM.)	APPEARANCE
Winter Sweet.....	P	12.1×7.2	7.744			Black, hard
	H†	○		10.0×6.0	1.562	Black, hard
	H‡	○		○		
Northern Sweet.....	P	12.0×7.4	7.350			Yellow, hard
	H	○		10.0×6.0	1.556	
Favorite Honey.....	P	11.0×7.2	5.040			Brown, hard
	H	○		9.8×6.2	2.075	Brown, hard
	P+H	10.8×7.2		9.3×5.7	1.490	Brown, hard
Coles Early.....	P	13.5×8.5	9.025			Black, hard
	H	○		10.4×5.5	1.345	Light yellow, soft
Early Kansas.....	P	14.0×8.8	12.120			Brown, hard
	H	○		14.0×5.8	1.100	Yellow, papery
Fordhook Early.....	P	13.0×8.0	10.105			Light yellow, hard
	H	○		9.2×5.4	0.830	Light yellow, papery
Iowa 5.....	P	13.5×8.0	9.120			Light yellow, hard
	H	○		8.5×5.0	0.708	Light yellow, papery
Selection 2.....	P	12.2×8.0	10.517			Black, hard
	H	○		10.2×6.4	1.725	Black, hard
Selection 5.....	P	○				
	H	○		9.8×5.9	1.178	Light brown to semi-hard
Stone Mountain.....	P	13.8×8.5	11.352			Yellow, hard
	H	○		8.7×4.9	0.300	Yellow, papery
Hawksbury.....	P	15.5×7.0	8.000			Black, hard
	H	○		8.0×4.0	0.275	Brown, papery
Yellow Melon.....	P	9.6×6.3	5.283			Dark brown, hard
	H	○		6.3×3.8	0.342	Light yellow, papery
Early Arizona.....	P	12.3×8.1	12.140			
	H	○				Black, hard

* Weights of seeds and seed coats calculated on air-dry condition and given in grams per 100 seeds from composite sample of 100-500 individuals.

† Composite sample of all treatments, of different concentrations and combinations.

‡ Seeds from these plants had been soaked in a 0.4 per cent colchicine aqueous solution.

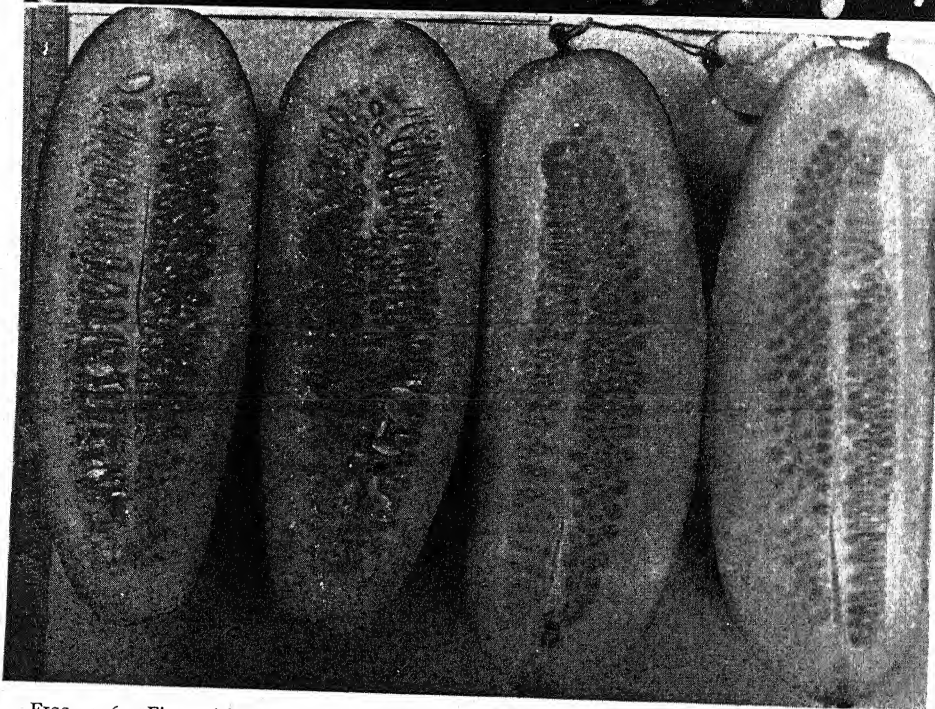
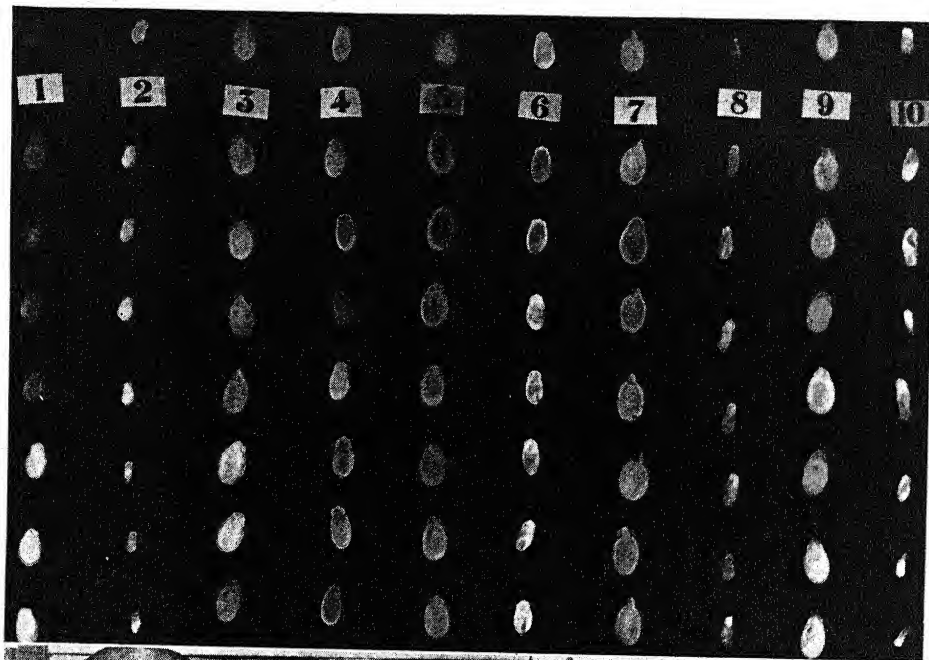
Honey, and Selection 2 produced what appeared to be normal seeds; however, none possessed an embryo. Yellow Melon, Fordhook Early, and Stone Mountain

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FIGS. 5, 6.—Fig. 5 (above), watermelon seeds: normal seeds from self-pollination as indicated in 1, 3, 5, 7 and seed coats from hormone treatment; 2, 4, 6, 8 in various varieties of watermelons listed below: 1, 2, Yellow Melon; 3, 4, Winter Sweet; 5, 6, Fordhook Early; 7, 8, Stone Mountain; 9, 10, Iowa 5. Note only empty seed coats, except for different degrees of development, in hormone-treated fruits. Fig. 6 (below), National Pickling cucumber: longitudinal sections of typical naphthaleneacetic acid-treated cucumber (at left) showing “undeveloped seeds,” compared in shape and size with open-pollinated cucumber (at right).

possessed only very small, papery seed coats. Pre-treatment of the seeds with a proper dosage (0.4 per cent for 4 days in Winter Sweet in this case) of colchicine prevented development of the integuments into seed coats in the parthenocarpic fruits. On the other hand, neither mixing the colchicine powder with the growth substance (K-naphthalene acetate) in the paste applied to the cut style of the blossom nor applying the substance to the flower produced from a stem which has been painted with colchicine paste prevented seed-coat development. Pre-treatment of the seeds with acenaphthene and other chemicals did not prevent development of empty seed coats.

EXPERIMENTS WITH CUCUMBER

In the American type cucumber, such as the National Pickling variety, a constriction of the stem end or blossom end is usually associated with seedlessness of

TABLE 8
NATIONAL PICKLING CUCUMBER: FRUIT SETTING AS RESULT
OF HORMONE TREATMENT; 1938

TREATMENT	NO. OF BLOSSOMS	NO. SET	PERCENT- AGE SET
2.5% naa in lanolin paste applied to cut style cap...	30	13	43
1% naa in lanolin paste applied to cut style cap....	32	24	75
5% naa in lanolin paste applied to cut style cap....	25	5	25
No treatment except nipped-off stigma.....	30	3	10
No treatment and no pollination.....	44	5	11
1% naa paste applied to stigma.....	11	6	54
Self-pollinated.....	15	8	53
Female flower sprayed with 0.05% naa solution with hand atomizer*	42	14	33

* This treatment used near end of season, which may account for low percentage of set.

that particular portion. Vegetative parthenocarpny seldom occurs in this variety (13, 14). An experiment was conducted with this variety in an attempt to secure seedless fruits of normal straight shape by means of growth substances. There were eight series of treatments. The treated blossoms were covered with wire cages before anthesis and left covered for 4-6 days after treatment. Naphthalene-acetic acid was the only hormone used. The results are given in table 8.

Although the number of samples was not large enough to permit any conclusive statement, the results indicate that naphthaleneacetic acid did induce parthenocarpic fruits of normal size and shape, either when the hormone was applied in lanolin paste of 1-5 per cent concentration or as a 0.5 per cent aqueous solution. Longitudinal sections of a self-pollinated seed-bearing cucumber and a stimulative parthenocarpic fruit are shown in figure 6. Hard viable seeds were present in the pollinated fruits; only soft, small, and undeveloped "ovules" were found in the parthenocarpic ones.

In the spring of 1939, under greenhouse conditions, about one dozen flower buds of the same variety were used in a further test. After the stigma and corolla were removed, the style cap was sprayed with naphthaleneacetic acid and trimethylamine in concentrations of 500 and 40 p.p.m., respectively. Three of the flowers were treated with 1 per cent naphthaleneacetic-acid paste. Straight fruits of normal size, having undersized seed coats without embryos, were obtained. One fruit which was produced by spraying with naphthaleneacetic acid at a concentration of 500 p.p.m. on April 26 was picked on June 19. It weighed 1132 gm. and was 26.5 cm. in length.

EXPERIMENTS WITH CUCURBITA PEPO

In the spring of 1939 an unnamed pumpkin of this species was used in the greenhouse. Four flowers were treated with acenaphthene and 1 per cent naph-

TABLE 9
EARLY PROLIFIC STRAIGHTNECK SQUASH: FRUIT AND CROP SETTING
AS RESULT OF HORMONE TREATMENT IN LANOLIN
PASTE TO CUT STYLES

TREATMENT	No. OF FLOWERS TREATED	FRUIT SET		CROP SET	
		No.	%	No.	%
1% kna.	3	3	100	3	100
2% kna*	10	10	100
1% kna and 0.5% c.	4	1	25	1	25
Check, cut style only.	2	0	0

* Blossoms treated very late in season and killed by first frost.

thaleneacetic-acid paste applied to the cut style on May 3. Three "fruits" reached the size of 5.0×4.9 cm., then turned soft and finally dropped on May 16. The two check flowers dropped when their ovaries had attained a size of 1.8×2.0 cm.

In the summer of 1939, eight varieties were used in further tests. The results with Early Prolific Straightneck and Dark Green Zucchini are shown in tables 9 and 10. The flowers were limited in number, but they indicated that both varieties are susceptible to parthenocarpic development. No external differences could be detected between normal and parthenocarpic fruits of squash. Although these two varieties belong to the same species, *Cucurbita pepo*, the integuments did not undergo the same degree of development in their parthenocarpic fruits. There were small, soft-textured seed coats in Early Prolific Straightneck but none in Dark Green Zucchini.

Other varieties used in this experiment, but with a more limited number of flowers (10-20 blossoms), were: Top of the Market, Hardin Bush, Omaha, Deli-

cata, Table Queen, and Fort Barthol. One mature fruit of Top of the Market was obtained by treatment with 2 per cent K-naphthalene acetate paste. No seeds or empty seed coats were found. One fruit of Hardin Bush was obtained from the same hormone treatment but dropped when it reached the size of 12.5×11.0 cm. Very little growth was observed in the treated ovaries of Fort Berthol, Omaha, and Delicata.

EXPERIMENTS WITH CUCURBITA MAXIMA

In the spring of 1939, Buttercup squash was grown in the greenhouse. The cut styles of a few flower buds were treated with acenaphthene, followed by 1 per cent naphthaleneacetic acid paste, and another group was treated with naphthalene-

TABLE 10
DARK GREEN ZUCCHINI SQUASH: FRUIT AND CROP SETTING AS RESULT OF HORMONE TREATMENT IN LANOLIN PASTE TO CUT STYLES

TREATMENT	NO. OF FLOWERS TREATED	FRUIT SET		CROP SET	
		No.	%	No.	%
1% kna.....	5	2	40	2	40
2% kna*.....	8	7	87
1% naa and 10% a.....	2	1	50	0
1% kna and 0.5% c.....	2	1	50	1	50
1% kna, 1% a, 1% iba, and 0.1% c.....	10	4	40	1	50
Check.....	2	0	0

* Blossoms treated very late in season and killed by first frost.

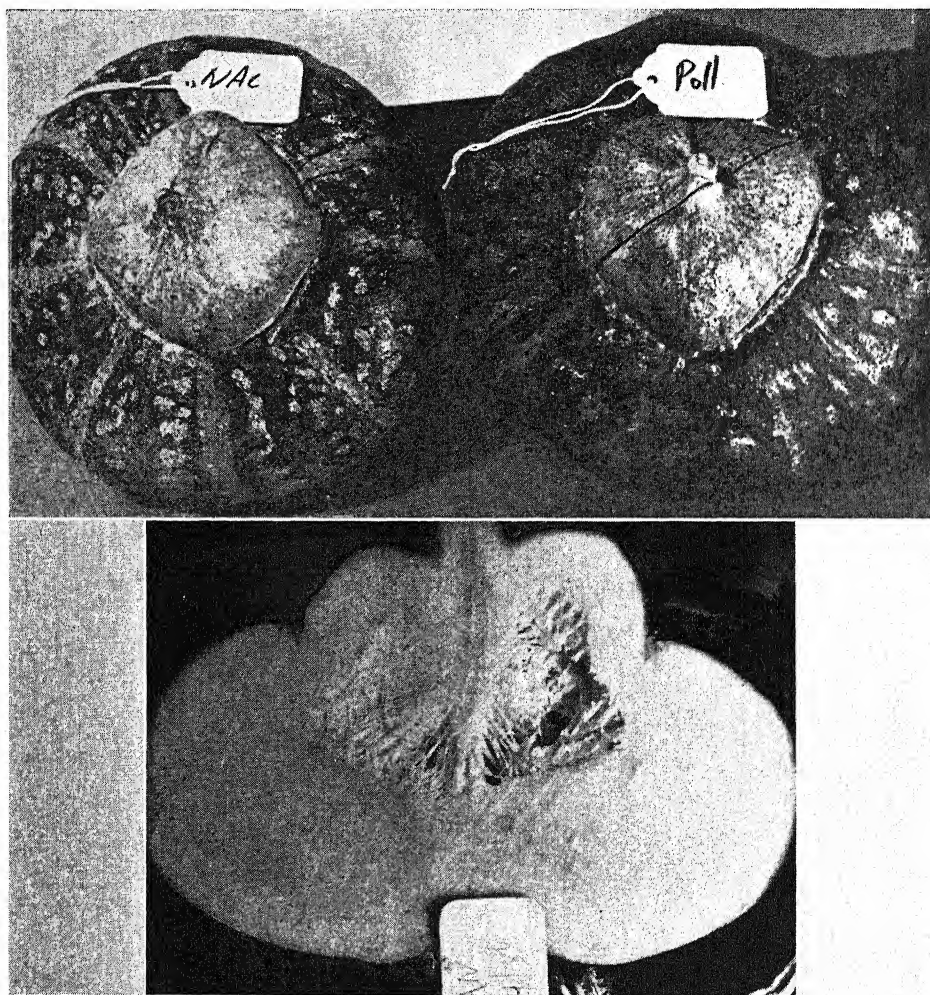
acetic acid alone. Flowers were treated on April 30 and the fruits matured about June 10. Seven externally normal fruits were produced (fig. 7), which were without seeds (fig. 8). The placental region of the parthenocarpic fruits was more restricted and they had a much thicker receptacle and pericarp than had normal seed-bearing fruits. Stylar tissue was fully developed. One undersized fruit was produced from treatment with 1 per cent naphthaleneacetic acid. In the summer of 1939 further experiments with the same variety were conducted. The results are shown in table 11.

EXPERIMENTS WITH CUCURBITA MOSCHATA

In the spring of 1939, the African Bell variety was used in the greenhouse. Out of four flowers treated with acenaphthene and 1 per cent naphthaleneacetic-acid paste, three normal fruits were obtained. One good fruit was also produced from naphthaleneacetic-acid treatment. The flowers were treated May 8 and fruits were picked July 6. One of them weighed 3270 gm. Fairly large seed coats, soft in texture, were present.

EXPERIMENTS WITH *CUCUMIS MELO* VAR. *RETICULATUS*

In the spring of 1939 about one dozen flowers of an unnamed selection of muskmelon were used and three mature fruits secured. In the summer of 1939, thirty-



FIGS. 7, 8.—Fig. 7 (above), Buttercup squash: blossom-end view of hormone-treated fruit (*nac*) and pollinated fruit (*poll*) showing similarity in external appearance. Fig. 8 (below), Buttercup squash: longitudinal section of hormone-treated fruit showing absence of seeds and seed coats. Placental region more restricted and with much thicker receptacle and pericarp than normal seed-bearing fruit.

five flowers of Honey Rock were treated with 1 and 2 per cent each of naphthaleneacetic acid and its potassium salt and a mixture of 1 per cent naphthaleneacetic acid and 10 per cent acenaphthene. Only negative results were obtained. A

limited number of flowers of cucurbits of unidentified species carrying U.S.D.A. Plant Introduction numbers were treated with various growth substances, but no parthenocarpic fruits developed.

EXPERIMENTS WITH SOLANACEOUS PLANTS

PEPPER (*CAPSICUM ANNUM*).—In the summer of 1938, various tests were made on the Harris Wonder variety. Twenty-four fruits out of twenty-five blossoms developed parthenocarpically as a result of treatment with 1 per cent naphthaleneacetic acid. Four fruits developed following spraying the stigmas with 0.05

TABLE 11

BUTTERCUP SQUASH: FRUIT AND CROP SETTING AS RESULT OF HORMONE TREATMENT IN LANOLIN PASTE TO CUT STYLES

TREATMENT	NO. OF FLOWERS TREATED	FRUIT SET		CROP SET*	
		No.	%	No.	%
1% kna.....	12	9	75	5	42
1% each of naa and iba...	7	1	14	0
1% naa and 10% a.....	9	2	22	0
1% naa and 10% a†.....	10	9	90	9	90
1% kna and 0.1% c.....	11	9	82	7	64
1% kna and 0.5% c.....	7	3	43	1	14
1% kna, 1% a, 1% iba, and 0.1% c.....	10	5	50	1	10
Check, cut style only.....	7	0	0	0

* By criteria of (a) loss of semi-glossy green color in rind and presence of yellow color near ground portion, (b) full size, and (c) toughness of rind.

† Application of abundance of acenaphthene to the cut style, then covering with naphthaleneacetic-acid paste. Treatment conducted in the spring of 1939 in the greenhouse.

per cent naphthaleneacetic acid. Out of seven flowers, the styles of which were cut but given no further treatment, four formed fruits which were seedless. Possibly parthenocarp in this instance may have been due to the stimulating effect of some wound hormone from the cut style or to vegetative parthenocarp. The treatment was started very late in the season, and all the plants were killed by frost before the fruits reached full maturity; however, all partly grown, hormone-treated fruits were seedless and normal in shape.

TOMATO (*LYCOPERSICUM ESCULENTUM*).—Fifteen flowers of the Michigan State Forcing variety were emasculated and treated with naphthaleneacetic acid. The results are presented in table 12. No seed developed. Most of the fruits were solid in the placental region.

Fruits resulting from an aqueous spray were much smaller than those produced from the paste treatment. Many of the parthenocarpic fruits showed internal breakdown at the blossom end, with the appearance of blossom-end rot.

EGGPLANT (*SOLANUM MELONGENA*).—Flowers of the variety New Hampshire Hybrid were treated late in the summer of 1939, and the fruits were still small when picked because of frost. The largest of the six parthenocarpic fruits reached a size of 11.5×10.2 cm. from treatment with a mixture of 1 per cent naphthaleneacetic acid and 10 per cent acenaphthene. Fruits from treatment with a mixture of 1 per cent each of naphthaleneacetic and indolebutyric acids were somewhat smaller. There appeared to be no external differences between pollinated and hormone-induced fruits, although the normally pollinated fruits grew more rapidly.

TABLE 12
TOMATO (MICHIGAN STATE FORCING): FRUIT SETTING AS
RESULT OF HORMONE TREATMENT

TREATMENT	No. OF FLOWERS TREATED	No. OF FRUITS SET	RANGE IN WEIGHT (GM.)
Sprayed with 500 p.p.m. naa solution..	9	9	33- 60
Sprayed with 50 p.p.m. naa solution. . .	3	3	42- 60
1% naa paste applied to cut style.	3	3	133-150

TABLE 13
STRAWBERRY: FRUIT SETTING AS RESULT OF HORMONE TREATMENT

TREATMENT	No. OF FLOWERS TREATED	No. OF FRUITS SET	REMARKS
Sprayed with 500 p.p.m. naa. .	12	12	Average over 20 mm. diameter, achenes small and embryoless
Self hand-pollination.	1	1	26 mm. diameter, achenes large with embryos
Open pollination.	5	2	22 mm. diameter, achenes without embryos
Check, emasculation only.	4	1	14 mm., lop-sided

On sectioning, only minute lines showed where the locules would normally have been, and there were no signs of ovules. The one control dropped within a week after the style was cut off.

EXPERIMENTS WITH STRAWBERRY

In the late fall of 1938, potted plants of an unnamed ever-bearing strawberry were used for fruit-setting experiments. About two dozen flower buds were treated. Unopened hermaphrodite flowers were first emasculated, then sprayed with naphthaleneacetic acid in concentrations of 50 and 500 p.p.m. with a hand atomizer. Parthenocarpic fruits of normal size containing achenes that appeared

normal were obtained; however, the latter were found to lack embryos. Some of the treated receptacles made only a slight initial growth, and achenes rarely developed on them. In the spring of 1939, a further experiment was conducted, the results of which are presented in table 13.

Summary

1. No parthenocarp, vegetative or stimulative, other than that due to hormone treatment, occurred in the watermelons and other species of *Cucurbita* tested, except for cucumber and pepper.
2. A very limited number of pollen grains did not cause the watermelon to set fruit. By adding a growth substance, however, mature fruits were obtained.
3. Treated watermelon flowers which did not develop and mature parthenocarpic fruits yet persisted on the vine, apparently because the substances prevented the formation of an abscission layer.
4. No apomictic seed of any sort developed in the parthenocarpic fruits produced from growth substances.
5. A mixture of two growth substances gave better results than one alone; for example, indolebutyric acid, acenaphthene, and sulfanilamide induced parthenocarp when employed alone, but gave more favorable results when mixed with other substances.
6. Indolebutyric acid (1-5 per cent), acenaphthene (10 per cent), and sulfanilamide (1.5 per cent) in lanolin paste failed to induce parthenocarpic development in watermelon.
7. Naphthaleneacetic acid had a greater effect on parthenocarpic development than its potassium salt and other growth substances.
8. There were great variations in fruit size and seed-coat development among the parthenocarpic fruits. Normal-sized hard seed coats were occasionally present in some fruits; however, seedless fruits of normal size which lacked seed coats were present in many species or certain varieties within the same horticultural group.
9. Pre-treatment of watermelon seeds with proper dosage of colchicine prevented development of integuments into seed coats on parthenocarpic fruits. Mixing the colchicine with a growth substance in the paste applied to the cut style did not prevent such seed-coat development.

This investigation was conducted at the Michigan State College, and the writer is grateful to Dr. R. P. HIBBARD of that Institution for supplying some growth substances for the preliminary trials; to Mr. H. L. SEATON for many of the plants in the earlier experiments; to Mr. K. C. BARRONS for his criticism of the manuscript; and especially to Dr. A. F. YEAGER for his constant encouragement and for making available facilities and opportunities to carry out this research. He is also

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LITERATURE CITED

1. BRITISH COLOR COUNCIL: Horticultural Color Chart. Vol. I. 1938.
2. FITTING, H., Die Beeinflussung der Orchideenbluten durch die Bestäubung und durch andere Umstände. Zeitschr. Bot. 1:1-86. 1909.
3. ———, Weitere Entwicklungsphysiologische Untersuchungen an Orchideenbluten. Zeitschr. Bot. 2:225-266. 1910.
4. GARDNER, F. E., and MARTH, P. C., Parthenocarpic fruits induced by spraying with growth promoting compounds. BOT. GAZ. 99:184-195. 1937.
5. GARDNER, F. E., and MARTH, P. C., Effectiveness of several growth substances on parthenocarpic in holly. BOT. GAZ. 101:226-229. 1939.
6. GUSTAFSON, F. G., Induction of fruit development by growth promoting chemicals. Proc. Nat. Acad. Sci. 22:628-636. 1936.
7. ———, Parthenocarpic induced by pollen extracts. Amer. Jour. Bot. 24:102-107. 1937.
8. ———, The cause of natural parthenocarpic. Amer. Jour. Bot. 26:135-138. 1939.
9. HAGEMANN, P., Ueber durch B-indolylessigsäure ausgeloste Parthenokarpie der Gladiole. Gartenbauwiss. 11:144-150. 1937.
10. HARTLEY, C. P., Injurious effects of premature pollination, with general notes on artificial pollination and the setting of fruit without pollination. U.S.D.A. Bur. Pl. Ind. Bull. 22. 1902.
11. MASSART, J., Sur la Pollination sans Fecondation. Bull. Jard. Bot. de L'état. Bruxelles 3: 89-95. 1902.
12. NIXON, R. W., and GARDNER, F. E., Effect of certain growth substances on inflorescences of dates. BOT. GAZ. 100:868-871. 1939.
13. SEATON, H. L., Relation of number of seeds to fruit size and shape in cucumbers. Amer. Soc. Hort. Sci. Proc. 35:654-658. 1937.
14. SEATON, H. L., HUTSON, R., and MUNCIE, J. H., The production of cucumbers for pickling purposes. Michigan Agr. Exp. Sta. Spec. Bull. 273. 1936.
15. WINGE, C., The chromosomes: Their numbers and general importance. Compt. Rend. Trav. Lab. Carlsberg 13:131-266. 1917.
16. YASUDA, S., On the behavior of pollen tubes in the production of seedless fruits caused by interspecific pollination. Jap. Jour. Genetics 8:239-244. 1933.
17. ———, The second report on the behavior of the pollen tubes in the production of seedless fruits caused by interspecific pollination. Jap. Jour. Genetics 9:118-124. 1934.
18. ———, Parthenocarpic caused by the stimulus of pollination in some plants of Solanaceae. Agr. and Hort. 9:647-656. 1934.
19. ———, Some contributions of the parthenocarpic caused by the stimulation of pollination. Bull. Sci. Fakultato Terkultura, Kjusu Imp. Univ. 7:34-55. 1936.
20. YASUDA, S., KOMATSU, T., and NONOMURA, T., Parthenocarpic caused by the stimulus of pollination in some plants of Solanaceae. Agr. and Hort. 5:287-294. 1930.

HYPERAUXINY IN CROWN GALL OF TOMATO¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 526

GEORGE K. K. LINK AND VIRGINIA EGGERS

(WITH ONE FIGURE)

Introduction

The literature contains several reports bearing on the relative auxin contents of healthy organs of tomato and of the same organs bearing galls incited by inoculation with the schizomycete *Phytophthora tumefaciens* (14, 16, 17, 18, 26, 27).

LOCKE, RIKER, and DUGGAR (17) compared noninoculated and inoculated plants, including tomato, as to epinasty of leaf petioles, initiation of adventitious roots, stimulation of cambium, inhibition of bud development, and delay of petiole abscission. They also compared the intensity of these responses following inoculation with avirulent and virulent strains of the parasite. It was concluded that these responses indicated an increase in growth substances in the inoculated plant and that it is less marked in those inoculated with the avirulent strain.

LINK, WILCOX, and LINK (14) prepared chloroform extracts of galls incited in tomato following inoculation with *P. tumefaciens*, and of control stems and leaves of tomato, and applied these to different parts of the axis of red kidney bean and tomato. Application of the gall extract through puncture in the bean hypocotyl was followed by whitening and local swelling. Application to the second internodes was followed by slight swelling and slight positive curvature. Extracts of control stems and leaves produced no effects. Application of the gall extract to hypocotyls of tomato seedlings led to slight negative bending and often to killing. In older tomato plants only killing was noted.

LOCKE, RIKER, and DUGGAR (18) used diffusion and the *Avena* technique (33) in testing the auxin content of noninoculated seedlings of tomato and of seedlings inoculated with *P. tumefaciens*. They made tests on the third, sixth, and ninth days after inoculation, using only one seedling of each kind on each of these days. On the basis of these limited tests they reported that the inoculated plants diffused more growth substance than the controls and that the differences increased with time. They also applied the diffusion technique to well-developed crown galls but could find no significant amount of auxin. They likewise failed to obtain any auxin when they assayed the chloroform and alcohol extracts of large galls.

Later RIKER, BERCH, and DUGGAR (27) reported—on the basis of extensive

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diffusion and extraction tests with segments of tomato plants bearing crown gall and with control segments of the same plant—that no significant differences in auxin contents exist between inoculated and control tissues on 1, 4, 8, and 16 days after inoculation. They conclude: “crown gall and control tissues are similar in relation to auxin obtained by extraction and diffusion.”

LINK, EGGERS, and MOULTON (16), in reporting on the preparation of plant tissues for auxin analysis by freezing and drying in vacuum and on success in demonstrating an auxinically more active extract from nodules of bean than from its roots, mentioned in passing that by use of material so prepared extracts of galls of tomato give a higher auxin assay than extracts of the control organ.

This paper reports the results of *Avena* coleoptile tests of extracts of non-inoculated hypocotyls of tomato and of hypocotyls of the same age and cultural conditions but bearing large crown galls produced by inoculation with pure cultures of *P. tumefaciens*. The first part of the paper is devoted to a comparison of extractants and of extraction procedures; the second, to comparative assays of noninoculated and inoculated organs.

Material and methods

Seed of tomato, variety Bonnie Best, was sown in soil in pots on September 17, 1940. Plants were thinned and selected for vigor and uniformity. On October 15, one half of the 786 plants available were set aside as controls, and the other half were inoculated in the hypocotyl with a pure culture of *Phytophthora tumefaciens* (Smith and Town.) Bergey *et al.* At this stage of development the hypocotyl had almost, but not completely, ceased elongation; each plant had two expanded leaves, and the internodes were beginning to elongate. The hypocotyl was selected for assay because it is the first organ to complete its elongation, and because its length and diameter are remarkably uniform in a population of plants grown under identical cultural conditions. The hypocotyls were inoculated with a special instrument, a micro-trident, with needles 4 mm. long and 1 mm. apart set firmly in a row. This insured that the triple puncture of each plant was identical as to spacing and depth. Before the puncture was made the inoculum was placed on the site of inoculation, which was midway between the ground line and the cotyledonary node. It was hoped that by using large populations representative samples would be obtained.

The hypocotyls were collected on November 5, 7, 11, and 14; that is, 20, 22, 26, and 29 days after inoculation. Each specimen was cut to include the portion between the ground line and the cotyledonary node. If adventitious roots were present, these were included in the sample. Control and inoculated hypocotyls were weighed and then frozen by placing them in pyrex tubes immersed in a mixture of cellosolve and dry ice. After the material was frozen solid it was cut into smaller

pieces while still frozen, placed in chilled pyrex flasks, and dried in the vacuum (16). After the material was dry it was ground in a Wiley micro-mill to pass 20, 40, and 60 mesh, thus insuring thorough mixing of the sample. The powder was weighed and then stored over P_2O_5 in vacuum desiccators. Dry weights were determined by oven drying at $100^\circ C.$ over night. They are given in table 1.

For each sample 100 plants were collected, except on the last day when only 43 each of noninoculated and of inoculated plants were available. Table 1 shows that while both inoculated and noninoculated hypocotyls were growing during the period of collection, the inoculated were enlarging more rapidly than the noninoculated, and that on the last two days the former on the average weighed al-

TABLE 1
NUMBER, WET WEIGHT, AND PERCENTAGE DRY MATTER OF NONINOCULATED AND
INOCULATED HYPOCOTYLS OF TOMATO

DATE OF COLLECTION	NONINOCULATED			INOCULATED		
	NUMBER	WET WEIGHT (GM.)	DRY WEIGHT (OVEN DRIED) (%)	NUMBER	WET WEIGHT (GM.)	DRY WEIGHT (OVEN DRIED) (%)
11/5/40.....	100	44	9.09	100	70.0	9.79
11/7/40.....	100	40	9.39	100	76.5	9.31
11/11/40.....	100	47	9.43	100	95.0	9.40
11/14/40.....	43	22.5	10.09	43	43.0	10.02

most twice as much as the latter. Practically all the growth at this time was diameter increase.

Figure 1 shows specimens exhibiting extreme difference as to length, diameter, root development, and gall diameter at time of collection. *A* and *B* are noninoculated, *C-E* are inoculated, and all are of the same age and cultural conditions. The cotyledonary node is indicated by a line drawn with india ink. In the main the inoculated hypocotyls differed from the noninoculated by being slightly shorter and slightly thicker, and by bearing more adventitious roots in addition to large tumors. These tumors were less green than the hypocotyl.

Since in preparation of the samples each hypocotyl was cut at the cotyledonary node, the coleoptile assays apply to whole noninoculated (plus such roots as they bore) as compared with whole inoculated hypocotyls (plus such roots as the hypocotyl and gall bore).

It was assumed that this is a better basis for sampling than use, on the one hand, of gall tissue alone and, on the other, of equal weights of contiguous tissue of the same hypocotyl, or of comparable tissue from a noninoculated hypocotyl, because it is not possible to define a sharp boundary of gall and non-gall

tissues. The abnormal increase in number of adventitious roots in the inoculated hypocotyl is clear indication that the auxin disturbance is not limited to the immediate site of the gall. The effect of inoculation with the parasite upon epinasty, adventitious root development, abscission of petiole, bud initiation, and cambial activity in regions remote from the site of inoculation and tumor development (14, 17, 18) indicates that if gall development is characterized by auxin disturbance (dysauxiny), this is not limited to the site of tumor production.

Since tests (16) with nodules and legume roots had indicated that dry ether extracts of thoroughly dry lyophilized material are auxinically inactive, and that

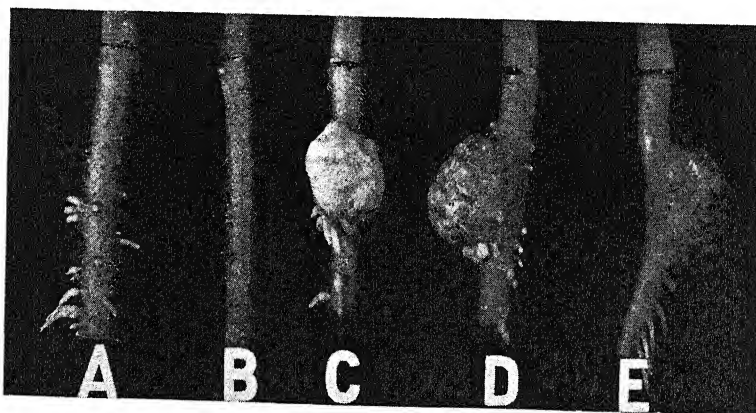


FIG. 1.—Tomato hypocotyls of same age (57 days) and cultural conditions. A, B, noninoculated; C-E, inoculated with *Phytomonas tumefaciens*. Photographed 29 days after inoculation. Natural size.

neither alcohol nor water alone is as effective an extractant as wet ether, this solvent was generally used. Similar results were reported by THIMANN and SKOOG (31) for oven-dried material. All ether used was distilled over wet FeSO_4 and CaO to eliminate peroxides. For experiments requiring the use of dry ether, the peroxide-free ether was first treated with CaCl_2 and then distilled and stored over metallic sodium. Other extractants and procedures are noted in the section on experiments and results.

After extraction, decanting, and filtration, the extracts generally were evaporated to dryness in a water bath at 50° – 60° C. and taken up in 1 cc. of 1.5 per cent agar, from which dilution series were prepared. The agar was cast into a block, volume 120 mm.³, which was then cut into twelve blocks of equal volume, 10 mm.³. Auxin assays were made by the standard *Avena* test (33) using an IDP time of 2–2.5 hours and a photographing time of 100 minutes. Each day the plants were calibrated with standard agar blocks containing indoleacetic acid, 0.015 and 0.030 mg. per liter of agar. The results of each test are given in arbitrary units, the unit being taken as that amount of auxin in 1 cc. of agar which gives the concen-

tration that produces 1° curvature of the *Avena* coleoptile under standard conditions. All units are calculated on the basis of 1 gm. of dry weight material (10 gm. wet weight) in 1 cc. of agar, and an average sensitivity of *Avena* coleoptile of 16° curvature for 30 γ indoleacetic acid per liter of agar. The data are based on tests with 10,484 *Avena* plants.

Experimental results

EXTRACTANTS AND EXTRACTION PROCEDURES

DRY ETHER AS EXTRACTANT AND SOLVENT.—Three samples, each 0.1 gm. dry weight of gall material (third collection), were treated with 50 cc. of dry peroxide-free ether by standing in the ether for 3 days in the icebox at 2° C. Each sample was decanted and filtered. The filtrate of one sample was evaporated to dryness, taken up in agar, and assayed. No activity was obtained. The powder was re-extracted for 3 days with 50 cc. of dry ether plus 1 cc. H_2O by standing in the icebox. The filtrate was evaporated to dryness, taken up in agar, and assayed. An activity of 210 units was obtained.

The dry ether filtrate of the second sample was evaporated and assayed. The extract was inactive. The powder was re-extracted and the filtrate evaporated as in the first sample. The residue was dried in an Abderhalden vacuum drier and then treated with dry ether which was filtered and evaporated. This residue was taken up in agar and assayed, to determine whether tomato auxin is soluble in dry ether. The activity was 105 units. To the dry ether filtrate of the third sample, 3 cc. of water was added. The water-ether filtrate was evaporated and the residue taken up in agar and assayed to determine whether a dry ether extract will become auxinically active upon addition of water. No activity was obtained.

The results of these tests with tomato tissue indicate (a) that after contact with thoroughly dry tissues, dry ether does not contain auxin; (b) that after contact with such material, wet ether does contain auxin; (c) that auxin from the hypocotyl is soluble in dry ether; (d) that presence of active auxin in the wet ether is not due to activation by water of a preauxin soluble in dry ether; but (e) that water itself in some way plays a role in rendering either active or potential auxin, or both, available for extraction by ether. These findings are in harmony with those previously reported for *Lemna* and other plants and for legume nodules and roots (31, 16). The results raise the question as to what happens to free auxin, that is, to the auxin present in fresh tomato tissue when these tissues are frozen and dried in vacuum. Possibly it is destroyed or it may be adsorbed and rendered nondiffusible by the freezing or the drying. At present we have no answer to these questions. THIMANN and SKOOG (31) report that drying may either destroy or fix auxin, or both, depending upon the auxin source.

WATER AS EXTRACTANT AND SOLVENT.—Since the experiments with dry and wet

ether indicated that water plays a role in extraction of auxins from tomato tissues, a few preliminary experiments were made to test the effectiveness of water as extractant and the role of the time factor. In the first experiment 0.1 gm. of dry powder of the gall material (third collection) was treated with 2 cc. of water for 24 hours. Equal volumes of the filtrate and of 3 per cent agar were mixed and assayed. An activity of 188 units was obtained.

In a second set of tests, 25 cc. of water was added to 0.1 gm. of powder of the inoculated hypocotyl (third collection) and set in the icebox at 2° C. for 3 hours. After filtration, the filtrate was frozen and evaporated to dryness in the vacuum system. The residue was taken up in wet ether, evaporated to dryness, and then taken up in 1 cc. of agar and assayed. An activity of 160 units was obtained. To a second 0.1-gm. sample of powder, 50 cc. of water was added and the sample set in the icebox at 2° C. for 6 days. The filtrate was frozen, dried in vacuum, and assayed. An activity of 720 units was obtained.

In this connection an experiment was run to determine whether ether effectively removes tomato auxin from water. To this end a 0.1-gm. sample of the gall material (third collection) was shaken for 3 hours at room temperature with 25 cc. of water and then shaken four times with four volumes of ether. The ether residue was filtered, evaporated, and the residue assayed. An activity of 216 units was obtained compared with 208 units for an identical sample shaken at the same temperature with 50 cc. of wet ether.

These results for tomato are in line with those of AVERY, CREIGHTON, and SHALUCHA (2), who report that water is an effective extractant of auxins of corn endosperm, and the report of THIMANN and SKOOG (31) that ether effectively removes *Lemna* auxin from water.

WET ETHER AS EXTRACTANT.—Wet ether has been most generally used as extractant of auxin and found best for *Lemna* (31) and for legume nodules and roots (16). To test its efficacy as extractant and the role of the time factor, 0.1-gm. samples of gall material (third collection) were extracted with 500 volumes of wet ether freshly distilled over wet FeSO_4 . In two instances, parallel extractions were made by placing one lot of the material on a shaker by the side of the material extracting by standing, to test the effect of constant agitation and hence presumably of better contact between extractant and powder used in these tests.

The results (table 2) show that relatively small volumes of wet ether are fully as effective extractants as equal volumes of water alone. The results also show that a relatively large amount of auxin is extracted in a short time, and that the amount increases slowly but significantly with lengthening time periods. The shaken samples showed an increase for the 24-hour but not for the 3-hour lot. This small increase and the other observations just made indicate that the slow liberation of auxin is not due primarily to the state of division of the material but

to a slow process which goes on in the presence of water. This is in harmony with the findings of THIMANN and SKOOG (31), who report that for *Lemna* an equilibrium probably exists between the water-plant material and the water-ether phases.

EXTRACTION BY SOXHLETIZATION

Two series of Soxhlet extractions with wet ether were run to test the effectiveness of this method. A preliminary test was made to determine whether the auxin fraction extractable from inoculated hypocotyls is affected by the temperature of boiling ether. To this end 100 cc. of wet ether was added to 0.2 gm. dry weight of the inoculated hypocotyl (third collection) and allowed to stand $3\frac{1}{2}$ days at 2° C. in the icebox. The filtrate was divided into two equal portions. The first

TABLE 2
AUXINIC ACTIVITY (IN UNITS) OF WET ETHER EX-
TRACTS OF 0.1-GM. DRY WEIGHT SAMPLES
OF INOCULATED HYPOCOTYL

TIME (HOURS)	ACTIVITY	
	WITHOUT SHAKING	SHAKEN
3.....	208	208
24.....	260	340
72.....	616
84.....	560
144 (a).....	600
144 (b).....	800

was evaporated and assayed and the second was boiled with ether in a reflux system for 24 hours and then evaporated and assayed. An activity of 560 units was obtained for each sample, indicating that the auxin fraction from tomato—like that of legumes (16)—is stable at the temperature of boiling ether. THIMANN and SKOOG (31) concluded that the auxin is destroyed by continued boiling in ether.

Another preliminary test was made—using dry ether—to determine whether the results obtained from standing in dry ether would apply. Tests were made with gall material from each of the four collections, and in each case the results were negative. Each sample, 0.1 gm. dry weight, was Soxhletized for 3 hours with dry peroxide-free ether.

Next, two sets of tests were made with wet ether on 0.1-gm. samples of gall material (third collection). The ether was not of the same lot for both sets and consequently probably did not contain the same amount of water. In the first set three samples were Soxhletized for 6, 12, and 24 hours, respectively, and in the

second set for 3, 6, and 12 hours, respectively; and after the first treatment, the 6-hour sample was extracted for another 6 hours with a new lot of ether and the 12-hour sample for another 12 hours with a new lot of ether.

The results are given in table 3, and indicate that Soxhletization with wet ether is a more efficient method of extraction of tomato material than standing in wet ether or in water, two 12-hour extractions yielding almost as much auxin as 144 hours of standing in water or in wet ether. THIMANN and SKOOG (31) report that the yields of auxin from *Lemna* by Soxhletization with ether were lower than those obtained by standing in wet ether in the cold. VAN OVERBEEK (32) reports

TABLE 3
AUXINIC ACTIVITY (IN UNITS) OF WET ETHER EX-
TRACTS OBTAINED FROM 0.1 GM. OF INOCULATED
HYPOCOTYL BY SOXHLETIZATION

TIME (HOURS)	ACTIVITY	
	FIRST EXTRACTION	SECOND EXTRACTION
1st series		
6.....	352
12.....	340
24.....	408
2d series		
3.....	168
6.....	520	0
12.....	624	40

that 24-hour Soxhletization of *Avena* coleoptile removes all auxin, including the active and the potential, which latter he considers a measure of the precursor present.

The yields for identical periods of extraction by Soxhletization and by standing in water or in wet ether varied considerably from test to test. This may be due to several factors, such as different pH, alone or together, with different amounts of water in the different ether lots or unequal initial and hence subsequent wetting of the lyophile powder.

COMPARATIVE ASSAYS OF AUXIN CONTENT OF NONINOCULATED AND INOCULATED HYPOCOTYLS

ASSAYS OF FOUR PAIRS OF SAMPLES COLLECTED 20, 22, 26, AND 29 DAYS AFTER INOCULATION.—For these assays 2-gm. samples (dry weight) were used. The materials collected 14, 20, 26, and 29 days after inoculation were tested in pairs, each pair consisting of noninoculated and inoculated hypocotyls for one date of collection. Freshly prepared peroxide-free ether (1000 cc.) was added to each sample

and allowed to stand in the icebox at 2° C. for 7 days. The extractant was decanted and filtered; the filtrate was evaporated to dryness, taken up in agar, and assayed. The results are given in table 4.

For each pair tested the gall extracts gave a markedly higher assay than the extracts of the noninoculated hypocotyl, the difference being least for the pair of the third collection. The differences are large enough to be significant, the ratios ranging from 1:5.4 to 1:15.

AUXIN ASSAY OF SUCCESSIVE EXTRACTIONS WITH WET ETHER.—There are many observations to the effect that a single extraction does not remove all auxin from

TABLE 4

AUXINIC ACTIVITY (IN UNITS) OF WET ETHER EXTRACTS OF NON-INOCULATED AND INOCULATED HYPOCOTYLS BY STANDING IN REFRIGERATOR IN DARK AT 2° C. FOR 7 DAYS; 2.0 GM. (DRY WEIGHT) OF MATERIAL AND 1000 CC. OF ETHER PER SAMPLE

DATE OF COLLECTION (1940)	AUXINIC ACTIVITY	
	NON- INOCULATED	INOCULATED
11/5, 20 days after inoculation.	38	215
11/7, 22 days after inoculation.	32	480
11/11, 26 days after inoculation.	80	440
11/14, 29 days after inoculation.	40	430

plant material (31, 16). The results recorded in the first section of this paper indicated that this applies also to the crown-gall material. To determine whether this applies to the noninoculated hypocotyl as well, and what amounts could be obtained in successive extractions, inoculated and noninoculated samples of the third collection were subjected to repeated extractions, beginning December 5, 1940. The last test recorded was made on July 24, 1941, after 209 days of extraction. Since the extracts were active on the last date of test, the extraction is being continued.

In the tests, 2 gm. each (dry weight) of the noninoculated and of the inoculated hypocotyl of the third collection (November 11, 1940) were covered each with 1000 cc. of freshly distilled peroxide-free ether and allowed to stand in the icebox at 2° C. for 7 days, with occasional shaking. The extractant was decanted, filtered, evaporated, and assayed (December 18). After the first assay the powders were dried and stored in a vacuum desiccator over P_2O_5 until January 3. They were then extracted for 24 hours each with 500 cc. ether. The extracts were decanted and tested on January 4 and the materials covered again with ether. After 7 days another assay was made (January 11). The procedure was repeated,

the periods of extraction varying from 4 to 42 days and the amounts of ether ranging from 250 to 500 cc. The results are given in table 5.

Throughout the 209 days of extraction, and on each of the seventeen successive extractions, the gall-bearing hypocotyl gave a higher auxin assay than the non-inoculated hypocotyls of the same age and cultural conditions. The amount of difference varied from extraction to extraction, the greatest contrast being noted

TABLE 5

AUXINIC ACTIVITY (IN UNITS) OF EXTRACTS OF NONINOCULATED AND INOCULATED HYPOCOTYLS THROUGH SUCCESSIVE EXTRACTIONS WITH WET ETHER BY STANDING IN REFRIGERATOR IN DARK AT 2° C.; 2.0 GM. (DRY WEIGHT) USED FOR EACH SAMPLE; 1000 CC. ETHER USED FOR FIRST EXTRACTION, THEN 500 OR 250 CC.

TREATMENT AND DATES OF TEST	AUXINIC ACTIVITY	
	Non-INOCULATED	INOCULATED
7 days in ether, 12/18/40.....	80	440
Redried powder kept in desiccator then in ether 24 hours, 1/4/41.....	0	74
7 days in ether, 1/11/41.....	30	450
4 days in ether, 1/15/41.....	26	120
7 days in ether, 1/22/41.....	30	80
7 days in ether, 1/29/41.....	40	100
7 days in ether, 2/5/41.....	12	100
7 days in ether, 2/12/41.....	26	76
6 days in ether, 2/18/41.....	14	32
8 days in ether, 2/26/41.....	14	61
7 days in ether, 3/5/41.....	16	48
7 days in ether, 3/12/41.....	10	40
15 days in ether, 3/28/41.....	19	72
21 days in ether, 4/17/41.....	16	44
22 days in ether, 5/9/41.....	11	38
42 days in ether, 6/20/41*.....	35	91
Total units.....	379	1866

* In a test on 7/24/41 the noninoculated assayed 20 units and the inoculated 62 units.

after the third extraction. During the first three extractions more than half the total obtained was extracted from the gall-bearing tissues, while less than one-third was extracted from the noninoculated hypocotyl. In this respect the gall material behaved like the legume nodule material (16, 31) and the noninoculated hypocotyl like roots of legumes, *Lemna*, and *Avena*. The two samples also differed as to rate and amount of swelling in water, the noninoculated swelling more rapidly and completely; as to amount of chlorophyll and other pigments liberated, the noninoculated yielding more chlorophyll; and as to extent and rate of browning, the noninoculated browning only slowly and slightly while the inoculated browned

rapidly and extensively, indicating a higher incidence of oxidizing enzymes for the latter, as reported by NAGY and RIKER (25). The second assay indicates that auxin liberation from the noninoculated hypocotyl is affected more deleteriously by drying than that of the inoculated.

Both samples are alike in that 209 days of extraction did not suffice to yield an auxin-free extract. In the light of GUSTAFSON'S report (7) that tomato seedlings yielded auxin after more than a year's extraction, these samples are likely to yield auxins for a long time. Unfortunately this means that the basis for developing a simple quantitative extraction method for this material is not yet at hand, because the total auxin content (active plus potential) cannot be determined.

SHORT EXTRACTIONS WITH WET ETHER.—In these tests 0.2 gm. (dry weight) of the noninoculated and 0.1 gm. (dry weight) of the inoculated hypocotyl were extracted with wet ether by standing for 5, 20, and 60 minutes, respectively, at 2° C. in the refrigerator. The extractants were decanted, filtered, and assayed. The noninoculated materials gave yields of 0 units in 5 and 20 minutes, and of 10 units in 60 minutes; the inoculated gave yields of 98 units in 5 minutes, 132 units in 20 minutes, and 145 units in 60 minutes.

WATER AS EXTRACTANT.—In this test 0.1 gm. each (dry weight) of the noninoculated and inoculated hypocotyl (third collection) were treated each with 2cc. of water for 24 hours by standing in the icebox at 2° C. Equal volumes of the filtered extractant were mixed with equal volumes of 3 per cent agar and assayed. The noninoculated hypocotyl yielded 38 units; the inoculated, 188.

YIELDS OBTAINED BY SOXHLETIZATION.—In the first experiment materials of the third collection were used, 0.2 gm. (dry weight) each being extracted for 6 hours in micro-Soxhlets and another pair for 12 hours. After extraction, the powder was placed in wet ether (50 cc.) for 7 days at 2° C. The extracts of the two series were made on different days with different lots of ether. The results are given in table 6. Comparative tests also were made with noninoculated and inoculated hypocotyls of the first and second collections. In these tests 0.5-gm. samples were extracted for 3 hours. After this the powders were extracted with wet ether for 24 hours at 2° C. in the icebox. The results are given in table 7. A comparative test was made for materials of the third collection by Soxhletizing it for 3 hours with dry ether. Neither inoculated nor noninoculated hypocotyls yielded an active extract.

CONTINUOUS ETHER EXTRACTION.—For this test, which supplements the Soxhlet extractions, 0.1 gm. each (dry weight) of the noninoculated and inoculated hypocotyl (third collection) were covered with 15 cc. of water in a continuous extractor and extracted for 1 hour each with ether. The extractants were filtered, evaporated, and assayed. The former yielded no measurable amount; the latter, 90 units.

These results support those for wet ether extracts to the effect that the extracts

of the inoculated hypocotyl are more active auxinically than are those of the noninoculated. Soxhletization proved the best short method for extraction of the noninoculated hypocotyl just as it has for the inoculated.

These observations may mean that the rate of liberation for auxin from the precursor (or bound form) is more rapid in case of the gall material, or that there

TABLE 6

AUXINIC ACTIVITY (IN UNITS) OF EXTRACTS FOLLOWING SOXHLETIZATION AND SUBSEQUENT EXTRACTION WITH WET ETHER

TREATMENT	Non- INOCULATED	INOCULATED
Soxhletized for 6 hours.	38	528
Resoxhletized for 12 hours.	33	220
Re-extracted by standing 7 days in wet ether.	18	509
Total.	89	1257

TABLE 7

AUXINIC ACTIVITY (IN UNITS) OF ETHER EXTRACTS AFTER SOXHLETIZATION AND SUBSEQUENT EXTRACTION WITH 50 CC. WET ETHER BY STANDING IN REFRIGERATOR AT 2° C. FOR 24 HOURS; 0.5 GM. (DRY WEIGHT) FOR EACH SAMPLE

DATE OF COLLEC- TION	TREATMENT	Non- INOCULATED	INOCULATED
7/5/41	3-hour Soxhletization.	0	18
	Re-extracted with wet ether for 24 hours by standing.	8	160
	Total.	8	178
7/7/41	3-hour Soxhletization.	0	24
	Re-extracted with wet ether for 24 hours by standing.	28	240
	Total.	28	264

is more auxin immediately available in the gall, or both. They may even mean that the auxins of the noninoculated material differ in solubility, equilibrium relations, methods of liberation, or in their initial sources (precursors) from the auxins of the inoculated materials.

EFFECT OF BOILING ON SUBSEQUENT AUXIN LIBERATION.—The preceding experiments show that lyophilized tomato materials do not yield auxin to dry ether,

even though the auxin is soluble in dry ether, but that wet ether extracts are auxinically active. This, together with the fact that fresh tomato material diffuses auxin into agar and yields auxin when extracted with wet ether, alcohol, or chloroform, means that freezing, or drying in vacuum, or both, destroy the active (diffusible and extractable) auxin present in fresh material, or that they fix or bind it to the treated tissues, as was reported for *Lemna* (31) and legume nodules and roots (16). It has been suggested by these investigators that water plays a role in liberating the fixed auxin by hydration.

The observation that many plant tissues over long periods liberate auxin in excess of the diffusible and initially extractable fraction suggests that water also plays a hydrolytic role in the process of enzymatic liberation of potential auxins from precursors. SKOOG and THIMANN (28) report that addition of proteolytic enzymes to a water suspension of *Lemna* material greatly accelerated the rate of auxin liberation. This suggests that if the enzyme systems, or the precursor involved, or both, could be destroyed without loss of the free auxin present, a single extraction might show the amount initially present. THIMANN and SKOOG (31) performed such experiments by treating *Lemna* leaves with steam, or by boiling, before extraction and found that this procedure did not affect the initial yield but stopped prolonged liberation of auxin. Their results indicate that an enzyme system is involved and that continued liberation of auxins is dependent upon bound auxin.

To determine whether these findings apply to noninoculated and inoculated hypocotyls of the tomato, some of our material was put to test by heating prior to extraction with wet ether. A preliminary test was made to determine the effect of boiling water upon the ether extracts of noninoculated and inoculated hypocotyls. A 0.5-gm. sample (dry weight) of the noninoculated (fourth collection) and a 0.1-gm. sample (dry weight) of the inoculated hypocotyl (third collection) were extracted by standing in wet ether for 3 days. Each extract was filtered and divided into two equal parts, one of which was evaporated and assayed while the other was evaporated, taken up in 0.5 cc. of water, boiled for 5 minutes, diluted with an equal volume of 3 per cent agar, and then assayed. The noninoculated material, unboiled, gave an activity of 25 units; the boiled, of 26 units; the inoculated material, unboiled, gave an activity of 581 units, and boiled, of 582 units—indicating that tomato auxin is not destroyed by boiling in water.

For the test itself a 0.5-gm. sample (dry weight) of the noninoculated and a 0.1-gm. sample (dry weight) of the inoculated hypocotyl (third collection) were placed in Soxhlet thimbles and immersed in 5 cc. of boiling water for 5 minutes, with stirring to prevent excessive clumping. The water extracts were shaken four times with four volumes of ether and the ether extracts filtered, evaporated, and assayed for auxins. The thimbles were dried in a stream of air and then placed in

the Soxhlet with wet ether for 24 hours. The extractant was filtered, evaporated, and assayed. The material then was given another extraction in the Soxhlet for 15 hours with wet ether, and a third one by standing in wet ether for 4 days at 2° C. Next each sample was incubated at 37.5° C. for 24 hours with 1 mg. of crystalline chymotrypsin per 0.1 gm. of material in water and a trace of toluene, adjusted to pH 9 with M/2 Na₂CO₃ (28). After filtration the pH was adjusted to 4 and the filtrate was extracted by shaking with four volumes of ether. The ether extract was evaporated and tested. The results are given in table 8.

The auxin recovered by the first Soxhletization is taken to be free auxin present at the moment the tissues were frozen. The inoculated hypocotyl contains more of this than the noninoculated. The absence of auxin in the extract of the second

TABLE 8

AUXINIC ACTIVITY (IN UNITS) OF NONINOCULATED, 0.5 GM., AND INOCULATED HYPOCOTYLS, 0.1 GM. (DRY WEIGHT), AFTER TREATMENT WITH BOILING WATER, WITH CHYMOTRYPSIN, AND WET ETHER

BOILING WATER EXTRACTION 5 MINUTES		THEN 24-HOUR SOXHLETIZATION WITH ETHER		THEN 15-HOUR SOXHLETIZATION WITH ETHER		THEN 7-DAY EXTRACTION STANDING IN ETHER		THEN 24-HOUR TREATMENT WITH CHYMOTRYPSIN AND EXTRACTION WITH ETHER	
NONIN- OCULATED	INOCU- LATED	NONIN- OCULATED	INOCU- LATED	NONIN- OCULATED	INOCU- LATED	NONIN- OCULATED	INOCU- LATED	NONIN- OCULATED	INOCU- LATED
0	0	10	70	0	0	0	0	10	20

Soxhletization and in the extract by standing in wet ether for 4 days is taken to signify that boiling destroyed either the agent by which, or the substance from which, potential auxin is liberated, or both. The presence of auxin in the extract following treatment with chymotrypsin is interpreted as meaning that boiling destroyed the enzyme which liberated auxin rather than the precursor, and that a proteolytic enzyme can liberate auxins from the cell constituents of higher plants.

The low yields of auxin, 10 and 20 units out of a possible 399 and 1858 units, respectively, for the noninoculated and inoculated hypocotyls, following digestion with chymotrypsin, indicates that (a) either the enzyme or the conditions during digestion were not favorable for maximum hydrolysis, or (b) most of the precursor had been altered by boiling so that auxin no longer could be liberated, or (c) proteins are not the only precursors of auxins. The toluene used to inhibit auxin formation by bacteria and fungi probably is not responsible for the low yields. In tests with agar containing extracts of the noninoculated hypocotyl with and without toluene, the former assayed 11.6 and the latter 11.4 units, indicating that the small amount of toluene used had affected neither the sensitivity of the coleoptiles nor the activity of the extract.

The fact that the amount of free auxin recovered from the inoculated hypocotyl is 70 units, while 98, 132, 145, and 720 units are obtained by extraction for 5, 20, 60 minutes, and 7 days, respectively, indicates that the gall at any moment contains a relatively large amount of free auxin which is liberated rapidly in extraction, but that the formation of auxin from its precursor proceeds slowly and at a decreasing rate.

The noninoculated hypocotyl behaves essentially like the inoculated, but differs quantitatively with a free auxin content of 20 units, no measurable liberation of auxin in 5 and 20 minutes, and a yield of but 10 units in 60 minutes and of 80 units in 7 days.

Discussion

The data show that extracts of the gall-bearing hypocotyls give higher free and potential auxin assays than the extracts of the normal and healthy hypocotyls. This holds for all pairs of samples tested and for all extractants and methods of extraction tried.

These findings are at variance with those reported by RIKER, BERCH, and DUGGAR (27). A comparative critique of the diametrically opposed data must await a further report by these investigators. The differences may be due to sampling methods, to nature of the samples, to methods of extraction, or to combinations of these. Extensive collections have been made to determine whether the findings reported here hold for other materials collected throughout the growing period of the tomato plant, and whether the gall material differs auxinically from contiguous tissue of the gall-bearing hypocotyl.

No diffusion tests have been made in the course of this investigation. Earlier ones conducted in this laboratory with old galls were negative, and LOCKE, RIKER, and DUGGAR (17, 18) report no success with diffusion tests on old galls. The basis for this failure possibly may lie in the same factor responsible for the observation by THIMANN and SKOOG (31) that while tomato callus grown in tissue culture yields auxin upon extraction, it does not liberate any by diffusion. They attribute this to disturbance in the polarity relations of the tumor tissues.

The fact that the gall tissues yield more auxin than the normal is in harmony with the findings of LINK, EGGERS, and MOULTON (16) that legume nodules yield more auxin than legume roots, and of MOULTON (24) that the smut galls of corn yield more auxins than comparable healthy tissues. It gives support to the hypothesis (12, 14, 33) that gall development, and in particular crown-gall development, is characterized by disturbed relations of growth substances (dysauxony), and that disturbed auxin relations (dysauxiny) induced directly or indirectly by wounding and by the parasite, through effects upon the host's metabolism, are part of the causal complex of crown-gall development. This hypothesis—if experimentally substantiated—gives support to the generally accepted corollary hypothesis that auxins play some role in the causal complex of normal and of

healthy growth. Any finding that abnormal auxin relations are not causally related to abnormal growth relations would seriously—but perhaps not fatally—weaken the auxin hypothesis for normal growth.

No conclusion can yet be drawn as to whether the hyperauxiny of the diseased hypocotyl is due (a) solely to auxins formed by the parasite; (b) solely to increased local auxin development by the host (with or without auxin increase in distal parts) and increased auxin transport to the site of gall production; or (c) to combinations of these. The senior writer has inclined from the beginning (14, 15) to the hypothesis that in addition to auxones, including auxins furnished by the parasite, the host cells—local and distant—also contribute to the hyperauxony of the affected organ. LEONIAN advanced the same view (11). Evidence for this interpretation is supplied by the observations of LOCKE, RIKER, and DUGGAR (17, 18) on the behavior of plant parts remote from the site of gall production.

The studies of NAGY and RIKER (25) on the chemical and physiological constitution of crown-gall tissue versus contiguous tissues corroborate and expand an earlier summary (26) to the effect that the chemical constitution of crown-gall tissue differs profoundly from that of healthy tissue, that the metabolism of the gall is at a higher level than that of contiguous tissues, and that the gall tissue approximates the chemical constitution and behavior of growing points.

The gall tissue is richer than the contiguous tissues in ash, ether extracts, total nitrogen, and simple forms of nitrogen. The polypeptide and protein fraction is two to four times greater in the gall than in the normal tissues. Glutathione and ascorbic acid are more abundant in the gall tissue; and the activity of oxidizing enzymes, catalase, peroxidase, and oxidase is higher. The tyrosinase activity of the gall tissue is 20–200 per cent greater than in the normal tissues. Our observations on the behavior of the noninoculated and inoculated tissues during extraction are in line with the reports regarding oxidizing enzymes. The finding that the polypeptide content of the galls is greater than that of the normal tissue is significant in that it is possible that amino acids and proteins are the precursors of auxins, and in that bacteria and fungi readily convert tryptophane to indoleacetic acid (33).

Biochemical studies of tumors induced by treatment with indoleacetic acid and similar auxins and with other auxones show that these pathic growths have much in common with tumors which are incited by parasites and are properly designated galls. Following the work of ALEXANDER (1) and COOPER (6) with indoleacetic acid and of STUART and MARCH (30) with indolebutyric acid, which showed that the local application of these substances not only mobilizes substances in distant parts of the plant but also leads to their transport and accumulation in the treated organ, and of BORTHWICK, HAMNER, and PARKER (4) which showed that proteins increase and nitrates decrease in the zone treated with indoleacetic acid and that there are changes in starch content, a series of researches

has established these findings in principle and in greater detail. MITCHELL and MARTIN (20) showed that in etiolated beans a local and systemic disturbance of digestion and translocation results upon application of indoleacetic acid. STUART (29) showed that there is abnormal movement of nitrogen and of carbohydrates to the site of treatment from distant parts following application of indoleacetic acid, and that there is increase in dry weight and of water, sugar, nitrogen, and calcium pectate in the treated zone. MITCHELL and HAMNER (21) and MITCHELL and STUART (22) also showed that the effects for indoleacetic acid are closely related to the concentration of the material applied. MITCHELL and WHITEHEAD (23) report that spraying of plants with auxins, including indoleacetic acid, is followed by increase in starch digestion. Similar results have been found by KRAUS and MITCHELL (10) and by MITCHELL (19) for substances such as alpha naphthalene acetamide and naphthaleneacetic acid, which are not known to occur in plants. These results and those of BEAL (3) with excised tissues indicate that local auxin and other auxone disturbances not merely mobilize materials in distant parts but also set up gradients or fields in movement and accumulation of materials which in some way are part of the causal complex of excessive local growth and development. Auxin apparently behaves not only as a mobilizer, but even more definitely as an incitant and focalizer of transport in the new gradients or fields which it establishes in the affected plant.

It has been reported (13, 14) that the crude auxin extract of *P. tumefaciens*, which BROWN and GARDNER (5) also had found to be active, gave the color test with ferric chloride, which is produced by indoleacetic acid. What auxin or auxins actually enter into the hyperauxiny of crown gall of tomato remains to be determined. HAAGEN-SMIT, LEECH, and BERGEN (8) have reported that an auxin extract from corn seed contains mainly indoleacetic acid and a small amount of pseudo-auxin a. This first report of the isolation of crystalline indoleacetic acid from green plant tissues weakens those criticisms in discussions of the crown-gall and legume nodule problems (9, 12, 14, 18) based on the fact that indoleacetic acid had not been isolated from green plants.

The questions whether the coleoptile tests measure the net effect of several auxins or even of auxins and of auxin inhibitors; whether the coleoptile test detects all growth substances (auxones); whether the auxin in crown gall is indoleacetic acid or indoleacetic acid plus other auxins; and lack of knowledge as to how these effects are brought about in the cells—these questions do not affect the fact that the coleoptile test reveals a greater content of growth-promoting substances in extracts of tomato crown gall than in extracts of the noninoculated hypocotyl. The finding at least shows that in one more respect gall tissue differs chemically and dynamically from normal healthy tissue of the same plant. These results, based on inoculation of an organ by a parasite but without decapitation, supplement and augment those auxin studies which utilize another line of pathological

evidence, that is, chirurgic mutilations in the form of decapitations of plants plus chemical infection in the form of applications of plant extracts and synthetic chemicals. These pathological techniques and data have to date furnished the main experimental basis for the physiological theory of growth substances.

Before a more successful approach can be made to the main problems still outstanding, among them estimation, isolation, and identification of the auxones involved in crown gall as compared with healthy tissues, and the role of these substances in healthy and in pathic growth, it is necessary to devise methods of extraction which are more quantitative than those available at present. None of the methods tried in these tests is satisfactory for determination of total auxins of tomato. Soxhletization with wet ether for 24 hours of material previously boiled for 5 minutes in water apparently suffices for removal of all free auxin. We have some evidence that a high pH of the extractant and of the tomato materials favors extraction by water and ether, and that digestion with proteolytic enzymes of materials freed from free auxins may provide a method for determination of the potential auxin.

Summary

1. The extraction of auxins from hypocotyls of tomato, noninoculated and inoculated with the gall-inciting schizomycete *Phytophthora tumefaciens*, has been studied.
2. Frozen vacuum-dried (lyophilized) tomato material does not yield auxins to dry ether. The immediately available or free auxin seems fixed until water liberates it.
3. Wet ether gave the best results as extractant, but even after seventeen successive extractions during 209 days, the process of auxin liberation had not reached completion.
4. Material boiled before extraction yields all its free auxin in one extraction by Soxhletization with wet ether for 24 hours, whereas non-boiled material continues to yield auxin. By combination of these methods the free auxin content may be distinguished from the potential or bound auxin. The latter may be a measure of auxin precursors, and is obtainable in part by digestion with chymotrypsin in the presence of toluene, from material freed from free auxin.
5. The inoculated hypocotyl yields both more free and more potential auxin than the noninoculated.
6. The significance of the finding that the growth disturbance known as crown gall is associated with disturbance in auxin relations (dysauxiny) is discussed with reference to the hypothesis that auxins play roles in normal and in healthy growth of plants and to the causal complex of gall development.

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LITERATURE CITED

1. ALEXANDER, T. R., Carbohydrates of bean plants after treatment with indole(3)acetic acid. *Plant Physiol.* 13:845-859. 1938.
2. AVERY, G. S., JR., CREIGHTON, H. B., and SHALUCHA, B., Extraction methods in relation to hormone content of maize endosperm. *Amer. Jour. Bot.* 27:289-300. 1940.
3. BEAL, J. M., Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. *BOT. GAZ.* 102:366-377. 1940.
4. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reaction of tomato plants to indoleacetic acid. *BOT. GAZ.* 98:491-519. 1937.
5. BROWN, N., and GARDNER, F. E., Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. *Phytopath.* 26:708-713. 1936.
6. COOPER, W. C., Hormones and root formation. *BOT. GAZ.* 99:599-614. 1938.
7. GUSTAFSON, F. G., Some difficulties encountered in the extraction of growth hormones from plant tissues. *Science* 92:266-267. 1940.
8. HAAGEN-SMIT, A. J., LEECH, W. D., and BERGEN, W. R., Estimation, isolation, and identification of auxins in plant material. *Science* 93:624-625. 1941.
9. KRAUS, E. J., Histological reactions of bean plants to l-tryptophane. *BOT. GAZ.* 102:602-622. 1941.
10. KRAUS, E. J., and MITCHELL, J. M., Histological and physiological responses of bean plants to alpha naphthalene acetamide. *BOT. GAZ.* 101:204-225. 1939.
11. LEONIAN, L. H., Review in *Phytopath.* 21:117-118. 1937.
12. LINK, G. K. K., Role of heteroauxones in legume nodule formation, beneficial host effects of nodules, and soil fertility. *Nature* 140:507. 1937.
13. LINK, G. K. K., and WILCOX, HAZEL W., Tumor production by hormones from *Phytomonas tumefaciens*. *Science* 85:126-127. 1937.
14. LINK, G. K. K., WILCOX, HAZEL W., and LINK, ADELINE DES., Responses of bean and of tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extracts, β indoleacetic acid, and wounding. *BOT. GAZ.* 98:861-867. 1937.
15. LINK, G. K. K., and EGGERS, VIRGINIA, *Avena* coleoptile assay of ether extracts of nodules and roots of bean, soybean, and pea. *BOT. GAZ.* 101:650-657. 1940.
16. LINK, G. K. K., EGGERS, VIRGINIA, and MOULTON, J. E., Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs: its extraction with dry ether. *BOT. GAZ.* 102:590-601. 1941.
17. LOCKE, S. B., RIKER, A. J., and DUGGAR, B. M., A growth hormone in the development of crown gall. (Abstract.) *Phytopath.* 27:134. 1937.
18. ———, Growth substance and the development of crown gall. *Jour. Agr. Res.* 57:21-40. 1938.
19. MITCHELL, J. W., Effect of naphthalene acetic acid and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plant. *BOT. GAZ.* 101:688-699. 1940.
20. MITCHELL, J. W., and MARTIN, W. E., Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants. *BOT. GAZ.* 99:171-183. 1937.
21. MITCHELL, J. W., and HAMNER, C. L., Stimulating effect of beta(3)indoleacetic acid on synthesis of solid matter by bean plants. *BOT. GAZ.* 99:569-583. 1938.
22. MITCHELL, J. W., and STUART, N. W., Growth and metabolism of bean cuttings subsequent to rooting with indoleacetic acid. *BOT. GAZ.* 100:627-650. 1939.
23. MITCHELL, J. W., and WHITEHEAD, MURIEL, Starch hydrolysis in bean leaves as affected by application of growth regulating substances. *BOT. GAZ.* 102:393-399. 1941.

24. MOULTON, J. E., Thesis for Doctor's degree. Univ. of Chicago. 1941.
25. NAGY, R., and RIKER, A. J., Some physiological studies of crown gall and contiguous tissues. Jour. Agr. Res. 57:545-556. 1938.
26. RIKER, A. J., and BERGE, T. O., Atypical and pathological multiplication of cells approached through studies on crown gall. Amer. Jour. Cancer 25:310-357. 1935.
27. RIKER, A. J., BERCH, HENRY, and DUGGAR, B. M., Growth substance in crown gall related to time after inoculation, critical temperature, and diffusion. (Abstract.) Phytopath. 31:21. 1941.
28. SKOOG, F., and THIMANN, K. V., Enzymatic liberation of auxin from plant tissues. Science 92:64. 1940.
29. STUART, N. W., Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. BOT. GAZ. 100:298-311. 1938.
30. STUART, N. W., and MARCH, P. C., Composition and rooting of American holly cuttings as affected by treatment with indolebutyric acid. Proc. Amer. Soc. Hort. Sci. 35:839-844. 1937.
31. THIMANN, K. V., and SKOOG, F., The extraction of auxin from plant tissues. Amer. Jour. Bot. 27:951-960. 1940.
32. VAN OVERBEEK, J., A quantitative study of auxin and its precursor in coleoptiles. Jour. Agr. Res. 28:1-10. 1941.
33. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

RESPONSES OF TWO SPECIES OF TOMATOES AND THE F_1 GENERATION TO SODIUM SULPHATE IN THE NUTRIENT MEDIUM¹

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Introduction

It has been recognized for some time that varieties of a given species of plant differ in their response to various environments. Inbred strains of corn and their F_1 generations have been found to vary in growth responses when available soil moisture was altered (13), when grown on rich and poor soil types (12), and when various fertilizer treatments were used (3). SMITH (20), using inbred strains and single crosses of corn, found marked differences in growth in relation to the phosphorus and nitrogen content in nutrient solutions. LYNES (15) used sand cultures and reported results essentially in agreement with those of SMITH. HARVEY (9) found a differential utilization of ammonium and nitrate nitrogen among inbred lines of tomato and corn with their hybrids, and BURKHOLDER and McVEIGH (2) found that inbred lines of corn and their hybrids differed in their responses to nitrogen supply in sand culture. Definite physiological symptoms of tomato plants in relation to high concentrations of Na_2SO_4 have been reported by EATON (6) and HAYWARD and LONG (11), using a commercial strain of the Marglobe variety.

The experiment here reported was designed to test the effects of high concentrations of sodium sulphate on (a) two species of tomato which are as completely homozygous as it is practicable to obtain, and (b) the heterozygous F_1 generation. Records were made of fruit production, anatomy of the stem, and growth responses.

Material and methods

The tomato, *Lycopersicum esculentum* Mill., variety Johannisfeuer, and *L. pimpinellifolium* (Jusl.) Mill., the Red Currant variety, together with the F_1 generation, were used. It is an interspecific cross, and the two species differ in many plant characters. The parent strains had been inbred under controlled conditions of pollination for at least five generations, and inasmuch as LINDSTROM (14) has found only 1-3 per cent of natural crossing under field conditions, they were considered essentially homozygous. POWERS (18), POWERS and LYON (19), and LYON (16) have reported the inheritance of some of the quantitative characters.

Seeds of each of the three lines were planted in flats containing sand on May 18,

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1940, and germinated in the greenhouse.³ Individual plants were transplanted on May 31 to 5-gallon glazed crocks filled with quartz sand containing 0.3 per cent of magnetite, which has been found (4) to provide sufficient iron for plant needs. The crocks were placed outdoors in large automatically operated nutrient culture tanks designed and described by EATON (4, 5). Eight plants were used in each tank, and the equipment provided 4800 liters of nutrient solution for each tank. This solution was circulated through the crocks for a period of 2 minutes during each hour from 6:00 A.M. to 6:00 P.M., with one additional irrigation at midnight. Each irrigation more than displaced the solution held in the crock. The plants were trained upright, and all axillary growth was pruned off twice weekly.

TABLE 1
COMPOSITION OF NUTRIENT SOLUTIONS

TREATMENT	P.P.M.		MILLIEQUIVALENTS PER LITER								
	B	MN	Ca	Mg	Na	K	HCO ₃	SO ₄	CL	NO ₃	H ₂ PO ₄
Tap water*.....	0.1	1.8	0.6	1.6	0.2	2.9	0.6	0.6	0.1
a) Base nutrient.....	1.0	0.2	6.2	4.6	1.6	3.4	2.9	4.6	3.0	8.1	0.6
b) Base nutrient+40 m.e. Na ₂ SO ₄	1.0	0.2	6.2	4.6	41.6	3.4	2.9	44.6	3.0	8.1	0.6
c) Base nutrient+80 m.e. Na ₂ SO ₄	1.0	0.2	6.2	4.6	81.6	3.4	2.9	84.6	3.0	8.1	0.6
d) Base nutrient+120 m.e. Na ₂ SO ₄	1.0	0.2	6.2	4.6	121.6	3.4	2.9	124.6	3.0	8.1	0.6

* Analyses of tap water kindly supplied by Dr. A. D. AYERS of the Salinity Laboratory. Ionic concentrations remained practically constant.

The nutrient solution was composed of KNO₃, MgSO₄, KH₂PO₄, H₃BO₃, MnCl₂, Na₂SO₄, CaCl₂, and Ca(NO₃)₂. Four solutions were used, consisting of (a) control solution which constituted the base nutrient, (b) base nutrient plus 40 milliequivalents per liter of Na₂SO₄, (c) plus 80 milliequivalents per liter of Na₂SO₄, and (d) plus 120 milliequivalents per liter of Na₂SO₄. Analyzed commercial salts were dissolved in tap water as used at Riverside, California. Salts were added in addition to those present in the water to raise the total concentration of the various ions of the solution to the desired strength (table 1).

To avoid injury of the seedlings immediately after transplanting and to assure the ripening and maturation of twenty fruits per plant, all tanks were initially supplied with base nutrient solution, and Na₂SO₄ was added as required in five equal amounts until the desired concentrations were attained. The additions extended over a 12-day period, and were made on June 27, July 1, 3, 6, and 9, 1940, when the plants were 40-52 days old and a few of them were in bloom. Additional water was

³ The seed was supplied through the courtesy of Dr. LEROY POWERS, Senior Geneticist, U.S. Horticultural Field Station, Cheyenne, Wyoming.

added to the reservoirs twice weekly to replace losses incurred through transpiration and evaporation. The pH of the nutrient solutions was checked daily with a Beckman pH meter and maintained at a pH of 5.5 by additions of HNO_3 when needed. The HNO_3 served the dual purpose of controlling the pH and maintaining nitrogen concentrations between 6 and 8 milliequivalents per liter (7).

After germination, the experiment was conducted outside, where a hygromograph placed near the plants recorded temperature and relative humidity. Pyroheliometer readings were obtained from the Citrus Experiment Station at Riverside, California, approximately 3 miles from the location of the experimental plots. The mean daily temperature from June 1 to September 15, 1940, was 74.0°F ., with a mean maximum of 92.9° and a mean minimum of 55.1°F . Highest temperatures were noted in July and August, with mean maximums of 96.7° and 96.0°F ., respectively. During the experiment the mean relative humidity was 68.3 per cent at 8:00 A.M. and 38.0 per cent at 12:00 A.M. The average daily total of solar radiation during this period was 579.4 gram calories per square centimeter of horizontal surface, with the highest values between June 15 and July 15.

The design of the experiment was that of a randomized block (8) with four treatments and twelve tanks. Three tanks were used for each treatment, with eight plants per tank. Eight plants of a strain were grown in each treatment, and the three strains were replicated in each tank. The three tanks, as well as the three strains of tomatoes within each tank, were randomized by the use of TIPPETT's randomization tables (22). The design provided for eight replications of each strain per treatment, with a total population of ninety-six plants. The data were reduced by means of the analysis of variance, and the t test (21) was used for determining whether particular differences were statistically significant. Odds as great as—or greater than—19.1 against the deviations, being due to the errors of random sampling, were accepted as statistically significant.

Experimentation and results

HEIGHT OF PLANTS

On August 13 the plants were 87 days old and had been growing on complete concentrations of their nutrient solutions for 35 days. At this time differences in plant heights were noted and recorded (table 2). There is a mean difference of 16.8 ± 6.57 cm. demonstrable between parental lines grown on the base nutrient solution, and the F_1 generation was 43.7 ± 6.89 cm. taller than the Johannisfeuer parent. Both of these differences are mathematically significant. The Johannisfeuer parent produced more growth in height of plant than the Red Currant parent, while the F_1 generation grew more rapidly than either parent. Although the magnitude of the differences between the strains, as well as the variability within them, are probably accentuated by pruning away all axillary growth, the relative

plant heights are in agreement with inherent differences demonstrated by POWERS (18).

The application of increasing concentrations of Na_2SO_4 does not affect the relative height of parental lines in comparable treatments or the phenomenon of heterosis shown in the F_1 generation. In a comparison of treatments *a* and *d*, significant mean differences of 17.9 ± 4.34 cm., 26.7 ± 6.63 cm., and 18.2 ± 5.87 cm. are demonstrable for Johannisfeuer, the F_1 generation, and Red Currant, respectively. Johannisfeuer plants grown in treatment *d* were 88.3 per cent as high as those in treatment *a*, while F_1 plants were 86.4 per cent as high, and Red Currant plants were 86.7 per cent as high on the basis of a similar comparison. For all

TABLE 2*
HEIGHT OF PLANTS IN CENTIMETERS

TREATMENT	STRAIN		
	JOHANNISFEUER	F_1 GENERATION	RED CURRANT
a) Base nutrient.....	153.3 ± 3.42	197.0 ± 5.98	136.5 ± 5.61
b) Base nutrient+40 m.e. Na_2SO_4	152.0 ± 2.52	191.6 ± 2.80	138.0 ± 3.29
c) Base nutrient+80 m.e. Na_2SO_4	142.7 ± 3.65	172.3 ± 2.71	126.5 ± 2.44
d) Base nutrient+120 m.e. Na_2SO_4	135.4 ± 2.67	170.3 ± 2.86	118.3 ± 1.73

*In any comparison, 14 degrees of freedom are available. When $t = 2.145$, $P = 0.05$ and when $t = 2.977$, $P = 0.01$.

practical purposes, the total growth depression produced by the addition of 120 milliequivalents of Na_2SO_4 to the base nutrient was the same in all three strains. If the concentrations of Na_2SO_4 had been applied earlier in the life of the plant, the magnitude of the differences might have been increased (11).

DRY WEIGHT OF PLANTS

On August 13, three plants of each strain grown in each nutrient concentration were selected for further experimentation. The selected plants were those which most closely approximated the strain mean for each treatment as regards plant height and date of first bloom. These plants were re-randomized and continued on their respective treatments. The remaining five plants of each strain in each treatment were harvested after all fruits, regardless of stage of development, had been picked. The root systems of each plant were washed successively through a series of mesh screens until separated from all sand particles. The vegetative parts (the fruits are not included) were dried at 80°C . and weighed (table 3).

It is possible to demonstrate in the data, mean differences of 35.2 ± 6.07 gm.,

95.4 \pm 11.20 gm., and 42.8 \pm 7.85 gm. for the vines of Johannisfeuer, F_1 generation, and Red Currant, respectively, when treatment *a* is compared with treatment *d*. The mean differences are well in excess of twice their standard error and are significant. In all strains the dry weight of the vines was less as the concentration of Na_2SO_4 in the nutrient solution was increased. When plotted against Na_2SO_4 concentrations, the dry weight of the vine closely approximated a linear regression in the case of the parental lines, while a comparatively pronounced reduction in dry weight was obtained for treatment *c* in the F_1 generation. The dry weight of vines in treatment *d* was 71, 69, and 56 per cent as great as in treatment *a* for Johannisfeuer, F_1 generation, and Red Currant strains, respectively. The effect

TABLE 3*

ANALYSIS OF DRY WEIGHT OF PLANTS GIVING STRAIN MEANS IN GRAMS
TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	JOHANNISFEUER		F_1 GENERATION		RED CURRANT	
	VINE	ROOT SYSTEM	VINE	ROOT SYSTEM	VINE	ROOT SYSTEM
a) Base nutrient...	121.0 \pm 4.64	19.4 \pm 2.12	308.2 \pm 7.67	62.9 \pm 3.67	97.8 \pm 6.10	18.0 \pm 2.61
b) Base nutrient + 40 m.e. Na_2SO_4 ...	106.6 \pm 4.90	16.5 \pm 1.22	276.4 \pm 7.06	57.3 \pm 2.01	83.8 \pm 2.60	18.7 \pm 0.63
c) Base nutrient + 80 m.e. Na_2SO_4 ...	101.0 \pm 5.77	15.1 \pm 0.68	220.2 \pm 10.48	45.7 \pm 2.64	71.4 \pm 3.57	18.0 \pm 0.67
d) Base nutrient + 120 m.e. Na_2SO_4 ...	85.8 \pm 3.92	14.9 \pm 0.67	212.8 \pm 8.16	42.2 \pm 2.73	55.0 \pm 4.94	18.7 \pm 0.66

* In any comparison, 8 degrees of freedom are available. When $t = 2.306$, $P = 0.05$ and when $t = 3.355$, $P = 0.01$.

of a high concentration of Na_2SO_4 is relatively greater on the Red Currant strain than it is on the other two in this respect.

In a similar comparison of root systems (table 3), a mean difference of 4.5 \pm 2.22 gm. and 20.7 \pm 4.57 gm. in dry weight is obtained for Johannisfeuer and the F_1 generation, respectively, between treatments *a* and *d*. Within these two strains, correlation coefficients computed between the dry weight of the root system and that of the vine were found to be highly significant. ($r = 0.73$, $t = 4.53$ for Johannisfeuer; $r = 0.80$, $t = 5.66$ for the F_1 generation. Nineteen degrees of freedom are involved in each line.) This means that within the limits of precision obtainable in this experiment, the root system and vine growth of Johannisfeuer and the F_1 generation were affected in the same way and to much the same degree by increases in Na_2SO_4 concentration. In the case of the Red Currant strain, no statistically significant differences in the dry weight of the root system produced were demonstrable. This phenomenon will be discussed later.

The expression of heterosis is clearly demonstrable. The dry weight of the F_1 generation vines is approximately 2.5 times that of the Johannisfeuer strain, which

is the heavier of the parents, while root systems are approximately 3.0 times as heavy in a similar comparison. The relationship is apparently constant in all treatments. This agrees with the observations and data of POWERS (18).

FRUIT PRODUCTION

When the plants were harvested, all fruits—regardless of size or state or maturity—were picked, counted, and weighed (table 4). At that time a few isolated fruits of the F_1 generation were completely red. Complete color change had not occurred on any fruit in parental lines.

TABLE 4*

ANALYSIS OF FRUIT PRODUCTION GIVING STRAIN MEANS
TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	JOHANNISFEUER			F_1 GENERATION			RED CURRANT		
	FRESH WEIGHT OF FRUIT (GM.)	NO. OF FRUITS	AVERAGE WEIGHT OF FRUIT (GM.)	FRESH WEIGHT OF FRUIT (GM.)	NO. OF FRUITS	AVERAGE WEIGHT OF FRUIT (GM.)	FRESH WEIGHT OF FRUIT (GM.)	NO. OF FRUITS	AVERAGE WEIGHT OF FRUIT (GM.)
a) Base nutrient.....	567 ± 58.7	30 ± 3.1	18.9 ± 0.39	286 ± 29.3	136 ± 7.9	2.1 ± 0.16	6.9 ± 2.00	45 ± 12.2	0.16 ± 0.016
b) Base nutrient + 40 m.e. Na_2SO_4	542 ± 63.8	34 ± 3.9	17.1 ± 2.93	174 ± 37.2	113 ± 12.9	1.5 ± 0.16	9.9 ± 2.30	65 ± 15.3	0.16 ± 0.013
c) Base nutrient + 80 m.e. Na_2SO_4	529 ± 79.7	36 ± 2.7	14.8 ± 1.49	144 ± 14.9	108 ± 6.7	1.3 ± 0.09	10.5 ± 1.49	73 ± 9.4	0.14 ± 0.008
d) Base nutrient + 120 m.e. Na_2SO_4	342 ± 61.8	33 ± 4.8	10.2 ± 0.88	105 ± 17.9	90 ± 11.8	1.2 ± 0.08	10.2 ± 2.66	81 ± 16.9	0.12 ± 0.012

* In any comparison, 8 degrees of freedom are available. When $t = 2.306$, $P = 0.05$ and when $t = 3.355$, $P = 0.01$.

The data for Johannisfeuer and the F_1 generation show mean differences in the fresh weight of the fruit produced of 225 ± 85.23 gm. and 181 ± 34.34 gm., respectively, when treatments *a* and *d* are compared. The differences are well in excess of twice their standard error and are significant. No statistically significant differences in this respect are demonstrable in the Red Currant strain. By comparing treatments *a* and *d*, the fresh weight of fruits produced by the Johannisfeuer are 40 per cent less as a result of the highest concentration of Na_2SO_4 used in the experiment. The fruit production of the F_1 generation was 63 per cent less in the same comparison. At every concentration of Na_2SO_4 , greater effects were observed on the F_1 generation. The most pronounced were observed between treatments *c* and *d* in the Johannisfeuer strain and between treatments *a* and *b* in the F_1 generation. In other words, there are indications that differences within as well as between strains exist for this character in its response to high Na_2SO_4 concentrations.

The data for the number of fruits produced by the F_1 generation show a mean difference of 46 ± 14.2 when treatments *d* and *a* are compared. In this strain the high concentration of Na_2SO_4 had an inhibiting effect on the number of fruits pro-

duced. No statistically significant differences were demonstrable in this respect for parental lines.

By again comparing treatments *a* and *d* in respect to the average weight of the individual fruit produced, mean differences of 8.7 ± 0.96 gm. for Johannisfeuer and 0.9 ± 0.18 gm. for the F_1 generation are demonstrated. Both differences are highly significant. The data for the Johannisfeuer strain suggest that the effects of increasing Na_2SO_4 concentrations on fruit size are at least additive and possibly cumulative, while the major effect in the F_1 generation occurs between treatments *a* and *b*, where a significant mean difference of 0.6 ± 0.23 gm. is demonstrable. In both cases, however, the average weight of the fruit at this stage was less when Na_2SO_4 concentrations were increased. No statistically significant trends are shown in the data for the Red Currant strain. Possible interpretations of these data will be discussed later.

Inherent strain differences in the fresh weight of fruits produced at this time are apparent, as a mean difference of 560.1 ± 58.73 gm. is demonstrable between parental lines in treatment *a*. No mathematically significant differences are found in the data between the mean of the F_1 generation and the arithmetic or geometric means computed from the means of parental lines. In treatment *a* the Johannisfeuer and Red Currant strains do not differ significantly in respect to the number of fruits set at this time in their life cycle, but the F_1 generation set approximately three times as many fruits as the Red Currant, and hybrid vigor is evident. A highly significant mean difference of 18.7 ± 0.39 gm. in the average weight of the fruit produced is evident between parental lines. The observed mean of the F_1 generation is significantly lower than the arithmetic mean (9.5 ± 0.20) computed from the means of parental lines.

MATURE FRUIT CHARACTERISTICS

The thirty-six plants (three replications, three strains, and four treatments) which had previously been selected for further experimentation on the basis of their uniformity and close approximation to the strain mean within treatments—in regard to plant height and date of first bloom—were grown until each plant had ripened twenty fruits. Each fruit was picked in the morning of the day that complete color change had occurred. Under these conditions, if the fruits are uninjured no detectable loss in weight occurs in the first 4 hours after harvest. Within this time limit each fruit was weighed in air to the nearest milligram and reweighed when immersed in distilled water. The weight in air is analogous to the mass (*M*) and the loss of weight in water is analogous to the volume (*V*). Fruit density (*D*) was computed by the equation $M/V = D$, using twenty fruits per plant and three plants of each strain in each treatment (table 5).

A mean difference in fruit density of 0.032 ± 0.0018 is indicated in the data for

parental lines in treatment *a*. The observed mean of the F_1 generation is significantly higher than either the arithmetic or geometric mean computed from parental strains, and partial dominance of the higher fruit density occurring in Red Currant is demonstrable. The relationship between strains is apparently constant in all treatments.

By comparing treatments *a* and *d*, mean differences in fruit density of 0.013 ± 0.0021 , 0.013 ± 0.0024 , and 0.017 ± 0.0012 for Johannisfeuer, F_1 generation, and Red Currant, respectively, were found. All differences are mathematically significant and the addition of 120 milliequivalents per liter of Na_2SO_4 to the base nutrient solution resulted in greater densities of mature fruits in all strains

TABLE 5*
ANALYSIS OF FRUIT DENSITY GIVING STRAIN MEANS
TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	STRAIN		
	JOHANNISFEUER	F_1 GENERATION	RED CURRANT
a) Base nutrient.....	0.996 ± 0.0015	1.025 ± 0.0019	1.028 ± 0.0010
b) Base nutrient+40 m.e. Na_2SO_4	1.003 ± 0.0001	1.026 ± 0.0018	1.038 ± 0.0011
c) Base nutrient+80 m.e. Na_2SO_4	1.005 ± 0.0023	1.032 ± 0.0028	1.039 ± 0.0007
d) Base nutrient+120 m.e. Na_2SO_4	1.009 ± 0.0014	1.038 ± 0.0014	1.045 ± 0.0007

* In any comparison, 118 degrees of freedom are available. When $t = 1.980$, $P = 0.05$ and when $t = 2.618$, $P = 0.01$.

tested. No differences between strains were noted in the percentage increase in fruit density as a result of treatment *d*. When treatments *a* and *b* are compared, significant mean increases of 0.007 ± 0.0015 and 0.010 ± 0.0015 are demonstrable for the Johannisfeuer and Red Currant strains, while no statistically significant difference is noted in the F_1 generation. In this experiment the fruits of the F_1 generation were affected less by the addition of 40 milliequivalents per liter of Na_2SO_4 to the base nutrient than were the fruits of the parental strains.

The data were examined in regard to the mean weight of each fruit produced by the strains when each fruit is picked immediately after complete color change (table 6). In Johannisfeuer and the F_1 generation, mathematically significant mean differences of 19.0 ± 4.72 gm. and 1.6 ± 0.44 gm., respectively, are demonstrable by comparing treatments *a* and *d*. In both strains the weight of each fruit is significantly less in treatment *c* than in *a*, but the addition of 40 milliequivalents of Na_2SO_4 produced no significant effect. There are no significant trends in the data for the Red Currant strain.

In this experiment, in comparisons made within strains no detectable effect of increasing Na_2SO_4 concentration was obtained for the number of days from planting to the date when twenty fruits per plant were ripened. The increased salt concentration did not significantly affect either the period in days from first fruit set to twenty fruits ripe per plant or the period in days from first fruit set to first fruit ripe per plant. When it is recalled that the concentration of the nutrient solutions was increased by additions of Na_2SO_4 at or near the date of first bloom, no effects would be expected, nor were they observed, in number of days from planting to date of first bloom. Within strains, the period from first bloom to first fruit set was unaffected by the treatments. In other words, under the conditions of this

TABLE 6*
ANALYSIS OF AVERAGE FRESH WEIGHT OF MATURE FRUITS GIVING
STRAIN MEANS TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	STRAIN		
	JOHANNISFEUER	F ₁ GENERATION	RED CURRANT
a) Base nutrient.....	57.6 ± 4.07	4.1 ± 0.35	0.84 ± 0.064
b) Base nutrient + 40 m.e. Na ₂ SO ₄	52.0 ± 3.58	4.9 ± 0.37	0.75 ± 0.056
c) Base nutrient + 80 m.e. Na ₂ SO ₄	42.1 ± 3.03	2.8 ± 0.33	0.80 ± 0.042
d) Basenutrient + 120 m.e. Na ₂ SO ₄	38.6 ± 2.39	2.5 ± 0.26	0.84 ± 0.031

* In any comparison, 118 degrees of freedom are available. When $t = 1.980$, $P = 0.05$ and when $t = 2.618$, $P = 0.01$.

experiment no detectable effects of increased concentrations on the rate of fruit development and maturation were observed.

The plants were harvested immediately after ripening twenty fruits per plant, and the dry weight of vines and root systems in all strains showed the same trends as in table 3. The dry weight of vines in all strains was less as the concentration of Na_2SO_4 was increased. The dry weight of the root systems produced by the Johannisfeuer strain and the F₁ generation was less as the concentration increased, but even at maturity no statistically significant differences in the dry weight of root systems in the Red Currant strain were demonstrable. As would be expected, the dry weight of the vines was greater in this harvest than in the earlier one, and the relative increase in dry weight of the vines was less as the concentrations of Na_2SO_4 increased. No statistically significant increase in the dry weight of root systems was shown in comparing data of both harvests. The magnitude of heterosis demonstrable in the dry weight of F₁ generation plants was less than in the previous harvest.

STEM ANATOMY

When each plant in the experiment was harvested, a section of the stem from the internode nearest the midpoint of the plant axis was fixed with Navashin's solution and air was evacuated from the tissues. The material was dehydrated in an ethyl-tertiary butyl alcohol series and infiltrated with a paraffin-beeswax-rubber mixture. Complete cross sections were cut at $15-30\ \mu$ and stained with a modified Flemming's triple stain. The material was mounted in balsam.

The stem sections of the plants which were harvested on August 13, as well as those harvested after twenty fruits were produced, had a continuous vascular cylinder, and comparatively few inner pericyclic fibers were thickened (10). The sec-

TABLE 7*
ANALYSIS OF STEM DIAMETERS GIVING STRAIN MEANS IN MILLI-
METERS TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	STRAIN		
	JOHANNISFEUER	F ₁ GENERATION	RED CURRANT
a) Base nutrient.	15.0 ± 0.47	14.2 ± 0.53	9.6 ± 0.31
b) Base nutrient + 40 m.e. Na ₂ SO ₄	13.3 ± 0.41	13.1 ± 0.34	9.1 ± 0.25
c) Base nutrient + 80 m.e. Na ₂ SO ₄	11.5 ± 0.38	12.4 ± 0.41	8.2 ± 0.23
d) Base nutrient + 120 m.e. Na ₂ SO ₄	10.7 ± 0.31	11.2 ± 0.31	8.2 ± 0.22

*In any comparison, 4 degrees of freedom are available. When $t = 2.776$, $P = 0.05$ and when $t = 4.604$, $P = 0.01$.

tions were magnified 27.5 diameters and the diameter of each stem measured. Four diameters were selected in order to divide the section into eight equal sectors, and the mean of the four measurements was used for each stem. The data in table 7 pertain to those plants harvested immediately after ripening twenty fruits.

Mean differences between treatments *a* and *d* of 4.3 ± 0.56 mm., 3.0 ± 0.61 mm., and 1.4 ± 0.38 mm. are demonstrable for the diameter of the Johannisfeuer, F₁ generation, and Red Currant strains, respectively. All differences are of sufficient magnitude to provide "P" values as small as, or smaller than, 0.05 and are statistically significant. In all lines there was less increase in diameter as the concentration of Na₂SO₄ increased. The percentage diameter of the stem as a result of the addition of 120 milliequivalents per liter of Na₂SO₄ to the base nutrient solution was 71.3 per cent for Johannisfeuer, 78.9 per cent for the F₁ generation, and 85.4 per cent for Red Currant. The last strain was affected less in this respect than was the Johannisfeuer, while the magnitude of the effect on the F₁ generation was intermediate.

The data for those plants harvested on August 13, and hence of comparable age rather than comparable maturity, were similar in most respects to those just cited and are not reported. Similarly, a smaller diameter of the stem was demonstrable in all strains as a result of the high Na_2SO_4 concentration. The data differed, however, in that the effect of increased salt concentration on the F_1 generation was greater than it was on parental lines.

At the increased magnification, the tissue systems were measured to the nearest millimeter on eight radii separated from one another by an angle of 45° . The mean of eight radial dimensions for each tissue system was used in the computation of an actual area. The actual area was computed using standard formulas, which were developed for perfect circles. The data in table 8 pertain to those plants harvested after twenty fruits per plant had ripened. The standard errors provide an estimation of variability between plants.

There are no statistically significant differences within a strain in the percentage of area of any of the tissue systems. The smaller diameter of the stem when the concentration of Na_2SO_4 is increased is a direct result of a correspondingly smaller area (and diameter) of each of the constituent tissue systems. For instance, the actual area of the xylem in a cross section of the stem of *Johannisfeuer* was less as the concentration in the nutrient solution was increased, but no significant differences were obtained in the percentage of the area of the xylem in the cross section of the stem.

In the cross section of the stem, a significant mean difference of 19.7 ± 3.71 per cent is demonstrable in the percentage of actual area covered by the pith between parental strains. The F_1 generation is intermediate between the parental strains in this respect, and differs significantly from both. The amount of cambial activity differs between parental lines when the formation of secondary xylem and phloem tissues is used as a criterion. A mean difference of 17.7 ± 3.37 per cent is shown for the amount of secondary xylem formed, and a mean difference of 3.8 ± 1.09 per cent is demonstrable for the secondary phloem. Both mean differences are statistically significant, and in both respects the F_1 generation is intermediate between parental strains. No significant difference is demonstrable in the percentage of area covered by the cortex. The cross section of the stem of the *Johannisfeuer* strain has a stele with a comparatively large amount of pith and small amounts of vascular tissue, while the stele of *Red Currant* has comparatively large amounts of vascular tissue and a small amount of pith. The stele of the F_1 generation is intermediate in all respects. The addition of increased concentrations of Na_2SO_4 to the nutrient solution does not alter inherent differences between genotypes in respect to the anatomy of stem sections.

The stems of the plants harvested when 87 days old showed the same anatomical relationships as those harvested after ripening twenty fruits per plant. The

TABLE 8*
ANALYSIS OF COMPOSITION OF STEM CROSS SECTIONS GIVING STRAIN MEANS IN PERCENTAGE OF ACTUAL
AREA TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	PERCENTAGE AREA													
	JOHANNISFEUER							F. GENERATION						
	PITH	XYLEM	PHLOEM	CORTEX	PITH	XYLEM	PHLOEM	CORTEX	PITH	XYLEM	PHLOEM	CORTEX	PITH	XYLEM
a) Base nutrient.....	43.5±3.28	27.7±3.19	5.7±0.30	19.3±0.51	34.3±2.09	36.2±1.19	7.4±0.61	20.1±1.31	23.8±1.73	45.4±1.07	9.5±1.05	17.3±1.20		
b) Base nutrient + 40 m.e. Na ₂ SO ₄	39.2±1.92	32.1±1.05	6.0±0.57	22.2±2.65	32.6±5.56	37.5±4.10	8.1±0.34	19.1±1.33	24.8±3.33	45.5±2.40	9.9±1.98	16.6±0.29		
c) Base nutrient + 80 m.e. Na ₂ SO ₄	44.0±2.50	29.1±2.14	6.0±0.31	18.6±0.78	32.7±0.50	38.1±0.38	6.1±0.37	18.9±0.79	24.0±1.96	48.5±1.55	8.8±0.61	17.0±0.71		
d) Base nutrient + 120 m.e. Na ₂ SO ₄	42.5±2.03	31.9±2.08	6.1±0.40	16.9±1.07	29.6±3.00	39.8±2.00	8.1±0.27	20.1±1.91	24.1±2.46	48.0±1.12	9.2±1.43	16.4±0.60		

* In any comparison, 4 degrees of freedom are available. When $t = 2.776$, $P = 0.05$ and when $t = 4.604$, $P = 0.01$.

magnitude of the differences was less at this date, but the differences were statistically significant. No effects of treatment were noted in the data.

Discussion

The growth depressions produced as a result of growing plants in nutrient solutions containing high salt concentrations have been pointed out (1, 17). EATON (6) and HAYWARD and LONG (11) have shown differential ionic effects at isosmotic concentrations of the nutrient solution. Unpublished data collected by EATON have indicated an interaction between a given salt concentration and such factors of the environment as temperature. It seemed logical that attention should also be given to the hereditary qualities of the test plant in addition to the preceding considerations.

Two criteria were used to measure the relative responses of the plants. First, at a given age, what is the response of the plant to varied environments with respect to any character? This method has been widely used in both physiological and genetic studies. Second, at comparable "physiological maturity," what is the response of the plants to varied environments in respect to any character? It was arbitrarily considered in this paper that plants were at comparable stages of physiological maturity when each had matured and ripened twenty fruits. The latter method has been less extensively used.

Where statistical significance is not demonstrable in an experiment (as is the case in the data for the Red Currant strain) in respect to mean weight of each ripe fruit produced, dry weight of the root system, total fresh weight of immature fruits, etc., it does not mean that differences do not exist. The use of larger populations and more refined methods might demonstrate significant differences in these characteristics. It may be assumed, however, that in this experiment Red Currant was less affected in these characteristics by high concentrations of Na_2SO_4 than were the other two strains.

The results of this experiment have shown that the presence of high concentrations of sodium sulphate in the nutrient solution affects the growth of all three strains. In many respects, the effects produced by the various concentrations on the strains were very similar. For example, the phenomenon of heterosis exhibited by the hybrid with respect to growth in height, dry weight production, number and weight of fruits, and so on, was exhibited to almost the same degree at every salt concentration used. In comparing the effects of increasing the concentration of sodium sulphate in the nutrient solution on the individual strains, it may be noted that Red Currant was affected less than were the other two with respect to such characters as dry weight of root system, fresh weight of all fruits produced, and average weight of mature fruit. For these characters the F_1 hybrid paralleled the Johannisfeuer parent more closely than it did the Red Currant. In all three

strains the density of the ripe fruit produced by plants subjected to increasing concentrations of sodium sulphate increased. Just what effect these changes in density might have upon the nutrient value of the fruit is not known. The effects of presence of sodium sulphate in the solution upon the internal anatomy of the stem of the three strains are very similar. In general there was no indication that the presence of sodium sulphate had a differential effect on any tissue system. In every case, the higher the concentration the smaller was the stem diameter; and in all stems the relative proportions of pith, xylem, phloem, and cortex remained the same, within the limits of error.

Anatomical results are not in entire accord with those of HAYWARD and LONG (11). There are, however, several differences in the methods of experimentation, such as: (1) the time at which the plants were subjected to the various concentrations in Na_2SO_4 , (2) ionic concentrations of nutrient solutions used, (3) pH of the solutions, (4) environmental factors, and (5) hereditary qualities of the plant. It is possible that future work may correlate the differences in the results obtained with one or more of these factors.

The occurrence of saline areas is general throughout western states. In addition, comparatively high concentrations of Na_2SO_4 and other salts are prevalent in water supplies used for irrigation purposes. To accompany proper soil management practices and more detailed studies of salt antagonism and toxicity effects, it may be possible and desirable to select and breed plants for tolerance to saline conditions. Such a program should eventually involve not only the production of strains with the characteristic of general tolerance to high salt concentrations but the actual selection and breeding of strains for specific concentrations of two or more ions.

Summary

Tomato plants of the Johannisfeuer strain and the Red Currant strain, together with the F_1 generation, were submitted to four treatments, providing four concentrations of Na_2SO_4 ranging from 4.6 to 124.6 milliequivalents of sulphate ion per liter of nutrient solution.

1. Plant height was less in all strains as the concentration increased. The growth depression resulting from the highest concentration was the same in all strains.
2. The dry weight of vines was less in all strains as the concentration increased.
3. The dry weight of the root system of the Johannisfeuer strain and the F_1 generation was less when the concentration was increased. No statistically significant reduction in this respect was obtained for the Red Currant strain.
4. The total fresh weight of immature fruits produced by plants 87 days old, as well as the average weight per fruit, was less in two strains when salt concentration was increased. No differences were noted in the Red Currant strain.

5. The number of immature fruits produced by each plant of the F_1 generation was less as the concentration increased.
6. The density of ripe fruits increased in all strains with increased concentration.
7. The mean weight of each ripe fruit of the Johannisfeuer strain and the F_1 generation was less with increased concentration.
8. The diameter of the stem of plants harvested after ripening twenty fruits, as well as of plants harvested at an earlier date, was less in all strains with increased concentration.
9. The smaller stem diameter was caused by an inhibited development of each of the component tissue systems.
10. Inherent differences between strains are discussed for each of these characters.

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LITERATURE CITED

1. BREAZEALE, J. P., Effect of the concentration of the nutrient solution upon wheat cultures. *Science* 22:146-149. 1905.
2. BURKHOLDER, P. R., and McVEIGH, I., Growth and differentiation of maize in relation to nitrogen supply. *Amer. Jour. Bot.* 27:414-424. 1940.
3. DE TURK, E. E., HOLBERT, J. R., and HAWK, B. W., Chemical transformations of phosphorus in the growing corn plant, with results on two first generation crosses. *Jour. Agr. Res.* 46:121-141. 1933.
4. EATON, F. M., Automatically operated sand-culture equipment. *Jour. Agr. Res.* 53:433-444. 1936.
5. ———, Plant culture equipment. *Plant Physiol.* 16:385-392. 1941.
6. ———, The toxicity and accumulation of chloride and sulfate salts in plants. *Jour. Agr. Res.* In press.
7. ———, Use of nitric acid in control of pH and nitrate levels in nutrient solution. *Plant Physiol.* In press.
8. FISHER, R. A., The design of experiments. Oliver and Boyd Co., London. 1937.
9. HARVEY, P. H., Hereditary variation in plant nutrition. *Genetics* 24:437-461. 1939.
10. HAYWARD, H. E., The structure of economic plants. Macmillan, New York. 1938.
11. HAYWARD, H. E., and LONG, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. *BOT. GAZ.* 102:437-462. 1941.
12. HOFFER, G. N., Some differences in the functioning of selfed lines of corn under varying nutritional conditions. *Jour. Amer. Soc. Agron.* 18:322-334. 1926.

13. KIESSELBACH, T. A., The comparative water economy of selfed lines of corn and their hybrids. Jour. Amer. Soc. Agron. 18:335-344. 1926.
14. LINDSTROM, E. W., Linked inheritance in tomatoes. Iowa State Coll. Jour. Sci. 1:3-13. 1926.
15. LYNESS, A. S., Varietal differences in the phosphorus feeding capacity of plants. Plant Physiol. 11:665-688. 1936.
16. LYON, C. B., Studies on the inheritance of the different stages of earliness in an interspecific cross between *Lycopersicum esculentum* Mill. and *L. pimpinellifolium* (Jusl.) Mill. Jour. Agr. Res. In press.
17. MAXIMOW, N. A., The plant in relation to water. New York. 1929.
18. POWERS, L., Studies on the nature of the interactions of the genes differentiating characters in a cross between *Lycopersicum esculentum* and *L. pimpinellifolium*. Jour. Genet. 39:139-170. 1939.
19. POWERS, L., and LYON, C. B., Studies on the inheritance of stages of development in crosses involving species and varieties of *Lycopersicum*. Jour. Agr. Res. In press.
20. SMITH, S. N., Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. Jour. Amer. Soc. Agron. 26:785-804. 1934.
21. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
22. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge University Press, London. 1927.

SEROLOGICAL STUDIES OF THE ERWINEAE I. ERWINIA AMYLOVORA

R. P. ELROD

Introduction

Erwinia amylovora (Burrill) Winslow *et al.*, etiological agent of fire blight, has been considered by some to be a well-defined species, yet others have found that various isolates often show numerous minor cultural and morphological differences. These dissimilarities have never been considered great enough to warrant new varieties, although they are often constant and readily distinguish one isolate from another (1, 11, 12, 16, 25, 19).

Serological methods have been employed with only moderate enthusiasm for bacterial plant pathogens, but enough work has been done to bring out the fact that certain species, such as *Phytophthora malvacearum* (E.F.S.) Bergey *et al.* (10, 26), *P. pisi* (Sacket) Bergey *et al.* (23), and *P. tabaci* (Wolf and Foster) Bergey *et al.* (21) are apparently serologically homogeneous, while others such as *P. tumefaciens* (E.F.S. and Townsend) Bergey *et al.* (20) and *P. stewarti* (E.F.S.) Bergey *et al.* (2, 15) are heterogeneous from an antigenic standpoint. In view of the increasing importance attached to the application of serological methods, it would be desirable to ascertain whether various isolates of *E. amylovora* might be distinguishable by agglutination, agglutinin-absorption, and also by precipitin tests, the last to be performed with carbohydrate materials extracted from the bacillary cells. A preliminary report of the present work has already been made (5).

Material and methods

The nine cultures¹ employed were obtained from widely separated localities and in the majority of cases from different hosts. The first four have been well discussed by ARK (1). His work gives the numerous differences detected in these isolates.

The sources of the nine isolates are as follows:

50.—Isolated from a stem canker on *Pyracantha angustifolia* at Berkeley, California, August, 1929.

55.—Isolated from a berry of *P. angustifolia* at Berkeley, January, 1932.

501.—Isolated from a twig lesion on *Crataegus oxyacantha* at Ithaca, New York, July, 1931.

SC.—Isolated from a twig lesion on *Pyrus communis* in South Carolina, June, 1925.

¹ The writer is indebted to Drs. P. A. ARK, E. L. WALDEE, E. M. HILDEBRAND, and S. B. DOOLITTLE for supplying some of the cultures used.

- 37.—Isolated from apple twig in Wisconsin, 1937.
84.—Isolated from *Crataegus monogyra* at Ames, Iowa, 1938.
83.—Isolated from *P. communis* at Ames, Iowa, 1938.
AM.—Obtained from Dr. E. M. HILDEBRAND, Ithaca, New York.
37, 83, 84, 85.—Obtained from Dr. E. L. WALDEE at Ames, Iowa.

Isolates 50 and SC were found by ARK to be only weakly pathogenic; all the others were moderately to strongly virulent. It was impossible for this writer to make virulence tests, but in view of ARK's observation that cultures of this organism will remain virulent after many years on stock agar, it can be assumed that in this test there were both virulent and avirulent isolates.

In the present work each isolate was streaked on nutrient agar, and from each plate a well isolated-smooth colony was picked and transferred to agar slopes containing nutrient agar having an agar concentration of 1.3 per cent. The growth from such a moist medium has been found to yield bacillary suspensions of high antigenic value. This growth was harvested with sterile saline solution and the cells were packed in an angle centrifuge and resuspended in saline in such a manner that the suspension contained approximately four billion bacteria per milliliter.

Rabbits weighing approximately 5 lb. were used. The following schedule of injections was employed: first day, 0.1 ml.; second day, 0.2 ml.; third day, 0.3 ml.; eighth day, 0.5 ml.; and thirteenth day, 1.0 ml. For the fourth and fifth injections new suspensions were prepared. All injections were made intravenously. The animals were bled by cardiac puncture on the eighteenth day and the sera preserved with merthiolate (1:10,000). Antisera were prepared for five of nine isolates. In all cases high agglutinating sera were obtained, the homologous titers ranging from 5,120 to 20,480.

Agglutination tests were set up in 0.5-ml. serial dilutions, beginning with 1:40 and carried past the titer of the serum, usually to 1:80,000. Antigen (0.05 ml.), freshly prepared as described, was added to each tube. The tubes were incubated in a waterbath for 4 hours at 50°–52° C. and placed overnight in the icebox (5° C.). The titer was determined as that tube with the highest dilution of serum which manifested clumping determinable by the naked eye. Saline and normal serum controls were used in each experiment. The latter was drawn from the rabbits before the beginning of the immunization series. In all cases controls were negative. Tests also were carried out, using as an antigen the primary suspension employed for the first injection, to which 1 per cent formalin had been added. No significant differences were noted except an enhancement in the degree of flocculation with the formalinized suspensions.

For the preparation of antigens for the absorption experiments, care was taken to select smooth colonies. These were picked into nutrient broth, and after 24 hours nutrient agar plates (1.3 per cent agar) were streaked heavily with a sterile

cotton swab which had been dipped into the broth culture. After 36 hours of incubation at room temperature, the growth was harvested by washing the organisms from the plate with sterile saline solution. The growth of three plates was combined and spun sharply in an angle centrifuge (3500 r.p.m.). After the cells were packed all excess moisture was drained from them. The quantity of packed cells was in the neighborhood of 0.25-0.4 ml. Ten ml. of anti-serum (1:20) was then added to the cells, the tubes were shaken until there was an even turbidity, and then incubated 1 hour at 37° C. in a waterbath. The suspension was centrifuged and the supernatant liquid decanted to another portion of packed cells.

TABLE 1
CROSS-AGGLUTINATION TITERS OF *ERWINIA AMYLOVORA* ISOLATES*

ORGANISM AGGLUTINATED	ANTI-SERUM PREPARED AGAINST				
	50	501	AM	55	84
50.....	5, 120	20, 480	10, 240	20, 480	10, 240
SC.....	2, 560	20, 480	5, 120	20, 480	40, 960
501.....	5, 120	20, 480	10, 240	10, 240	20, 480
55.....	5, 120	10, 240	20, 480	20, 480	20, 480
AM.....	10, 240	20, 480	10, 240	20, 480	20, 480
37.....	10, 240	10, 240	20, 480	10, 240	20, 480
84.....	5, 120	10, 240	10, 240	10, 240	20, 480
83.....	10, 240	10, 240	5, 120	10, 240	20, 480
85.....	5, 120	10, 240	10, 240	10, 240	10, 240

* Saline and normal serum controls negative.

Usually it was necessary to repeat the procedure four times. The fourth absorption was incubated 2 hours at 37° C. and then placed overnight in the icebox. After centrifuging, the supernatant was diluted to 1:40. Further serial dilutions were made as found necessary.

Experimental results

AGGLUTINATION TESTS

Agglutination tests were performed with the five sera, using suspensions of the nine isolates as antigens. Table 1 shows the high degree of antigenic homogeneity. The homologous titers ranged from 5,120 to 20,480, and the cross-agglutination titers were virtually as high. In some cases the latter dropped one tube below the homologous titer, as for example culture SC (2,560) in anti-50 serum (homologous titer 5,120), and in some cases they advanced to as great a degree (the homologous titer anti-AM serum was 10,240; the heterologous 55 was 20,480). It would be impossible, however, to distinguish one isolate from another on the basis of these reactions.

The type of agglutination was extremely variable. All the isolates proved to be peritrichously flagellate, as stained by the method of LEIFSON (14). This flagellation was emphasized by the usually typical floccular type of clumping. On occasion, however, this type of agglutination was less evident and the somatic or granular type was predominant. The addition of 1 per cent formalin to the antigen tended to stabilize the type of agglutination and usually enhanced the floccular type.

That this high degree of cross-agglutination was specific for *E. amylovora* was brought out by cross-agglutination tests performed with four cultures of *E. tracheiphila* (E.F.S.) Holland (isolates 71A, 72A, 73B, and 75C), one of *E. salicis* (Day) comb. nov. (isolate 80), one of *E. lathyri* (Manns and Tabenhaus) Magrou

TABLE 2
CROSS-AGGLUTINATION TITERS WITH UNRELATED ORGANISMS*

ORGANISM AGGLUTINATED	ANTI-SERUM PREPARED AGAINST				
	50	501	AM	55	84
71A.....	<40	160	80	40	160
72A.....	<40	< 40	< 40	<40	< 40
73B.....	<40	< 40	< 40	<40	< 40
75C.....	<40	160	160	40	80
80.....	<40	80	< 40	40	80
BL.....	<40	< 40	< 40	<40	< 40
MY.....	<40	< 40	< 40	<40	< 40
20 strains soft- rot organisms	<40	< 40	< 40	<40	< 40

* Saline and normal serum controls negative.

(isolate BL), one of *E. ananas* Serrano (isolate MY), and twenty strains of organisms of the soft-rot group. As can be seen in table 2, cross-agglutination occurred in only a small percentage of the tests. Whether or not the cross-reactions of certain strains of *E. tracheiphila* and *E. salicis* are only a unilateral relationship has not been ascertained. This relationship will be examined in subsequent experiments. In no case, however, did these titers approach the homologous reactions, and for the most part did not exceed 1 per cent of the homologous titer.

AGGLUTININ-ABSORPTION EXPERIMENTS

The results of the agglutinin-absorption experiments are recorded in table 3. All five sera were absorbed with each of the nine isolates, forty-five absorptions in all. In each case a complete removal of homologous and heterologous agglutinins resulted. It may be assumed, therefore, that at least five of the isolates (50, 501, 55, AM, and 84) are serologically identical (those for which anti-sera were pre-

pared), and that the other four contain at least those fractions common to the preceding five. Whether or not they possess other antigenic factors could be determined only after preparing anti-sera for them. There is no doubt, however, that the nine isolates are closely related, if not serologically identical.

The experiments were controlled for specificity by absorbing each of the sera with an unrelated organism (for example, *E. tracheiphila* or *E. salicis*). In no case were any agglutinins removed, either homologous or heterologous.

PRECIPITIN TESTS

Since DOCHEZ and AVERY (4) discovered substances in pneumococcus filtrates which were later shown to be carbohydrate in nature (9), bacterial polysaccharides have become an important factor in problems concerned with bacterial classifica-

TABLE 3
ABSORPTION EXPERIMENTS

ANTI-SERUM	ABSORBED BY	RESIDUAL AG- GLUTINATION AGAINST ALL STRAINS
50.....	50, 501, 55, AM, SC, 83, 84, 85, 37	Nil*
501.....	501, 50, 55, AM, SC, 83, 84, 85, 37	Nil
55.....	55, 501, 50, AM, SC, 83, 84, 85, 37	Nil
AM.....	AM, 501, 50, SC, 83, 84, 85, 37, 55	Nil
84.....	84, AM, 501, 50, SC, 83, 85, 37, 55	Nil
50, 501, 55, AM, and 84.....	<i>E. tracheiphila</i> or <i>E. salicis</i>	All to titer (table 1)

* Nil = less than 1:40.

tion. Specific carbohydrates have been isolated from many groups of bacteria. Such work with the *Salmonella* group (7, 8), hemolytic streptococci (13), and other forms have been landmarks in the progress of bacteriology.

In view of the success attained in other groups, it was deemed advisable to ascertain whether the isolates of *E. amylovora* could be distinguished one from another by means of precipitin tests, using extracted carbohydrate materials as antigens. Previous precipitation experiments with phytobacteria have employed filtrates of broth cultures. Such an antigen certainly cannot always be considered specific but will contain carbohydrates and protein as well as metabolic products.

The method used for extraction was that of FULLER (6). It was found admirable for the purpose, although it was usually shortened by eliminating the acid-alcohol step. The material thus obtained gave a strongly positive Molisch test for carbohydrate and a weak or negative Biuret reading for protein. For the precipitin tests the material was serially diluted and ring tests employed in the usual

manner. The tests were read after 1 hour of incubation at room temperature. The titers were all consistently the same (1:100,000).

As shown in table 4, it was impossible to distinguish one isolate from another by means of direct precipitin tests. It is possible that absorption experiments with carbohydrate material might detect differences. Absorption with whole cells resulted in negative results, however, even as had the agglutinin-absorption experiments. Carbohydrates derived from *E. salicis* (isolate 80) and *E. tracheiphila* (isolate 75C) were negative in all immune sera. This was likewise true of saline controls. The various *E. amylovora* antigens failed to precipitate in normal serum.

TABLE 4
PRECIPITIN TESTS WITH EXTRACTED CARBOHYDRATES

ANTIGEN (1-100,000)	ANTI-SERUM PREPARED AGAINST					NORMAL SERUM
	50	501	AM	55	84	
50.....	+	+	+	+	+	-
501.....	+	+	+	+	+	-
AM.....	+	+	+	+	+	-
SC.....	+	+	+	+	+	-
55.....	+	+	+	+	+	-
83.....	+	+	+	+	+	-
84.....	+	+	+	+	+	-
85.....	+	+	+	+	+	-
37.....	+	+	+	+	+	-
<i>E. salicis</i> (1:100).....	-	-	-	-	-	-
<i>E. tracheiphila</i> (1:100).....	-	-	-	-	-	-
Saline.....	-	-	-	-	-	-

Discussion

In general, the bacteriologist has had little success in attempting to link source of isolation, virulence, physiology, and morphology with definite serological results. In the realm of phytobacteriology, BUSHNELL and SARLES (3) noted that cross-inoculation experiments and cross-agglutination experiments with root-nodule bacteria could not be correlated. This had been previously found to be true by STEVENS (24). Likewise, BRAUN and McNEW (2, 15) found that differences in serological properties could not be correlated with virulence or colony type in *Phytomonas stewartii*. SHARP (18) concluded that two strains of *Bact. flaccumfaciens*, although differing in virulence, type of colony produced, and appearance on agar slopes, were not distinguishable by agglutination experiments. This was also true of *Bact. phaseoli* (18).

The results presented in this paper show that serologically *E. amylovora* is a homogeneous species, and that no groups are demonstrable which might be cor-

related with source of isolation, morphology, and physiology. In this case, however, instead of there being several serological groups in which cultural and morphological differences overlap, we are dealing with a serologically constant group, in which there is wide variance in morphology, physiology, and pathogenicity (1).

The precipitation experiments reported in this paper were not concerned with showing a group relationship, but were an attempt to isolate a specific carbohydrate. STAPP (22) contends that when a positive precipitation test is obtained the identity of the species is confirmed, no matter what the cultural and morphological characteristics might be. This is in all probability true with *E. amylovora*, but offers no advantages over the more easily performed agglutination test. Inasmuch as there are no means of differentiating the isolates serologically, one from another, it is impossible to conclude whether the carbohydrate material is group or type specific in nature. That it could be one or the other is shown by the fact that in *Salmonella* (8), carbohydrate material derived from the bacillary cell acts in the same manner as the somatic components, that is, they are group specific. In *Proteus* (17) such carbohydrate material confers type specificity to the organism.

An absolutely serologically homogeneous species of bacteria is not in the least common. Numerous well-defined species often segregate themselves into many serological groups. Among the bacterial plant pathogens, *Phylomonas tumefaciens* (20), *P. stewartii* (2, 15), and others act in this manner. It is difficult to understand why *E. amylovora* should show such homogeneity, although it is true that certain other phytoacteria show similar serological characteristics (10, 26, 23, 21), but not in the striking manner demonstrated by *E. amylovora*.

It may be that *E. amylovora* represents the ultimate in a definite evolutionary chain, and that owing to a specialized pathogenic action within a limited host range the organism has become uniform. The arguments against such being the case are numerous and the idea is presented only as a possibility. For the supposition to obtain credence it would be necessary to demonstrate other links in the chain. None is at present known. The fact that my cultures had been carried in stock for many years might account in some way for this homogeneity. Perhaps isolates taken directly from the field would show some antigenic variation. This is only a bare possibility, however, for many organisms closely related serologically (for example, *Salmonella*) do not become serologically one after years in stock.

Summary

1. Nine isolates of *Erwinia amylovora*, obtained from five different localities and from as many different hosts, were compared serologically.
2. On the basis of agglutination tests and agglutinin-absorption experiments, no antigenic difference among any of the nine isolates could be detected.

3. Carbohydrate materials extracted from the bacillary cells and used in precipitation tests likewise failed to differentiate the organisms.

4. It is concluded that serologically *E. amylovora* is an exceedingly homogeneous species.

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LITERATURE CITED

1. ARK, P. A., Variability in the fire-blight organism. *Phytopath.* 27:1-28. 1937.
2. BRAUN, A. C., and McNEW, G. L., Agglutinin-absorption by different strains of *Phytomonas stewartii*. *BOT. GAZ.* 102:78-88. 1940.
3. BUSHNELL, O. A., and SARLES, W. B., Investigations upon the antigenic relationships among the root-nodule bacteria of the soybean, cowpea, and lupine cross-inoculation groups. *Jour. Bact.* 38:401-410. 1939.
4. DOCHEZ, A. R., and AVERY, O. T., The elaboration of specific soluble substance of pneumococcus during growth. *Jour. Exp. Med.* 26:477-493. 1917.
5. ELROD, R. P., and STARIN, W. A., Serological studies of *Erwinia amylovora*. (Abst.). *Jour. Bact.* 41:87-88. 1941.
6. FULLER, A. T., The formamide method for the extraction of polysaccharides from hemolytic streptococci. *Brit. Jour. Exp. Path.* 19:130-139. 1938.
7. FURTH, J., and LANDSTEINER, K., On precipitable substances derived from *Bacillus typhosus* and *Bacillus paratyphosus B.* *Jour. Exp. Med.* 47:171-184. 1928.
8. ———, Studies on the precipitable substances of bacilli of the *Salmonella* group. *Jour. Exp. Med.* 49:727-743. 1929.
9. HEIDELBERGER, M., and AVERY, O. T., The soluble specific substance of pneumococcus. *Jour. Exp. Med.* 38:73-79. 1923.
10. HORGAN, E. S., The value of serological tests for the identification of *Pseudomonas maltovarum*. *Jour. Bact.* 22:1181-1184. 1931.
11. HOWARD, F. L., Do significant physiological strains of *Bacillus amylovorus* Burr. Trev. exist? *Iowa Acad. Sci. Proc.* 36:105-110. 1929.
12. JONES, D. H., Bacterial blight of apple, pear and quince trees. *Ont. Agr. Coll. Bull.* 176. 1909.
13. LANCEFIELD, R., A serological differentiation of human and other groups of hemolytic streptococci. *Jour. Exp. Med.* 57:571-595. 1933.
14. LEIFSON, E., A method of staining bacteria flagella and capsules together with a study of the origin of flagella. *Jour. Bact.* 20:203-211. 1930.
15. McNEW, G. L., and BRAUN, A. C., Agglutination test applied to strains of *Phytomonas stewartii*. *BOT. GAZ.* 102:64-77. 1940.
16. PIERSTORFF, A. L., Studies on the fire-blight organism, *Bacillus amylovorus*. N.Y. (Cornell) *Agr. Exp. Sta. Mem.* 136. 1931.
17. PRZESMYCKI, F., Analyse éléments antigènes des souches du *Proteus* HX₁₉, et X₁₉O. *Compt. Rend. Soc. Biol.* 95:744. 1926.
18. SHARP, C. G., Virulence, serological, and other physiological studies of *Bact. flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*. *BOT. GAZ.* 83:113-144. 1927.

19. SNOW, L. M., A new host for the fire blight organism, *Baccillus amylovorus*. *Phytopath.* 12: 517-524. 1922.
20. STAPP, C., Der bakterielle Pflanzenkrebs und seine Beziehungen zum tierischen und menschlichen Krebs. *Ber. Deutsch. Bot. Ges.* 45:480-504. 1927.
21. ———, Bakterielle Tabackrankheiten und ihre Erreger. *Angew. Bot.* 12:241-274. 1930.
22. ———, Contemporary understanding of bacterial plant diseases and their causal organisms. *Bot. Rev.* 1:405-425. 1935.
23. ———, Der bakterielle stengelbrand der Erbsen. *Zentralbl. Bakt.* 96:1-17. 1937.
24. STEVENS, J. W., Can all strains of a specific organism be recognized by agglutination? *Jour. Inf. Dis.* 33:557-566. 1923.
25. STEWART, V. B., The fire-blight disease in nursery stock. N.Y. (Cornell) *Agr. Exp. Sta. Bull.* 329. 1913.
26. WILLIAMS, O. B., and GLASS, H. B., Agglutination studies of *Phylomonas malvaceara*. *Phytopath.* 21:1181-1184. 1931.

HISTOLOGICAL STUDIES ON THE ROOT OF *MELILOTUS ALBA*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 527

FRANCES R. BOTTUM

(WITH FOURTEEN FIGURES)

Introduction

The distribution, soil adaptation, habits, and agricultural value of biennial white sweet clover, *Melilotus alba* Desv., have been extensively investigated, a notable survey being that of LLOYD (7). Studies of the relative growth rates of roots and tops have been made by SNIDER and HEIN (11), WILLARD (15), and MARTIN (9). MARTIN has also described the germination and establishment of the seedling. The habit of growth of the root system has been investigated by WEAVER (14). COOPER (1), GUIGNARD (4), WATT (13), and YOUNG (20) have given accounts of the embryogeny; and the cytology of the meristematic cells of the root tip has been investigated by ELDERS (2). McMURRY and FISK (10) have reviewed those points in earlier studies of seedling structure in the Leguminosae by DE CANDOLLE, VAN TIEGHEM, NÄGELI, GERARD, and COMPTON which are of significance in the study of *Melilotus*, and they have described the transition of root to stem structure in the seedling.

The object of this investigation is to supplement these studies with a description of further details of the root structure, especially of the older roots.

Material and methods

The plants used in this study were grown in the field, except very young seedlings for which pots of soil or germination dishes were used. The seed coats were softened by treatment with concentrated sulphuric acid, employing the method of LOVE and LEIGHTY (8), except that the time was shortened from 15 to less than 5 minutes.

Inoculation of soil with nodule-forming bacteria was insured by mixing the seeds, before sown, with fresh soil taken from around the roots of healthy older plants growing elsewhere. Plants were collected at convenient intervals throughout the period of growth.

Whole seedlings and short pieces of older roots were killed and fixed in formalin-acetic-alcohol or in Navashin's solution. The methods of clearing seedlings and free-hand sections in chloral hydrate or in cedar oil, of maceration, and of imbed-

ding in paraffin were used. Xylol was the clearing agent employed, and sections of roots were made at 8 or 12 μ . They were stained with Flemming's triple stain or with safranin and fast green.

Observations

PRIMARY ROOT OF SEEDLING

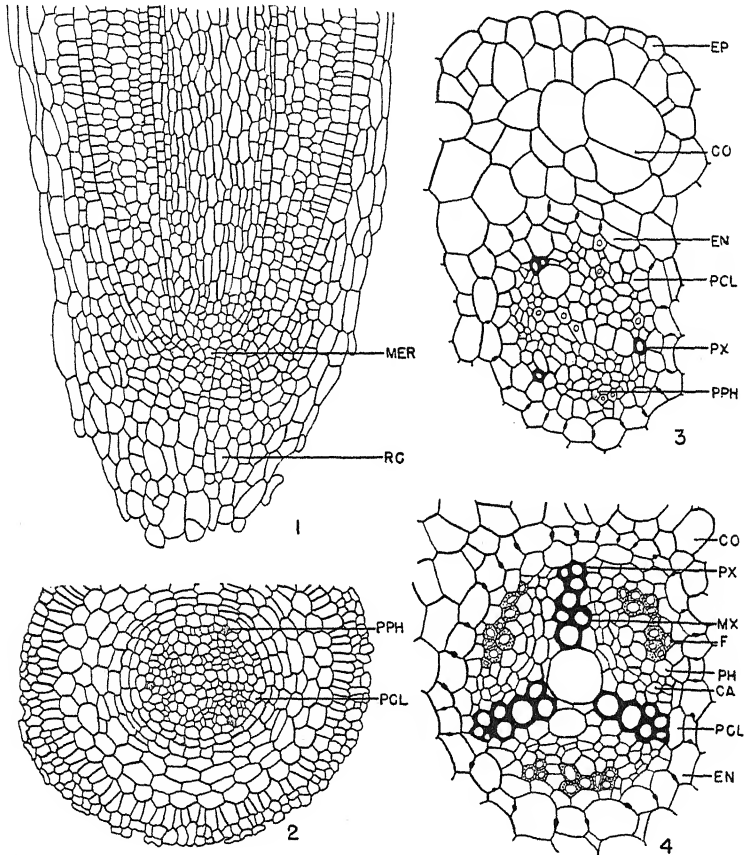
In the embryo of the seed, according to MARTIN (9), the radicle, hypocotyl, cotyledons, plumule, and even the crown buds are recognizable. In germination of the seed, the primary root on emerging penetrates the soil and forms a slender unbranched tap root. The ontogeny of the primary root corresponds to the fourth angiospermous type as described by JANCZEWSKI (5). Growth is accomplished by the activity of a transverse plate of meristem from which the stele, cortex, and central cone of tissue of the root cap develop. A lateral meristem, continuous near the root tip with the terminal meristem, gives rise by radial and periclinal divisions of the cells to the outer portion of the calyptra, and proximally constitutes a distinct dermatogen (fig. 1).

The root cap is composed of closely packed cells with conspicuous nuclei and relatively dense cytoplasm, except in the outer layers of cells which are separating from the cap. The young root immediately proximal to the terminal meristem shows a central region of small cells, varying in size, surrounded by one or two sheaths of larger cells, many of which are undergoing conspicuous tangential division and are densely cytoplasmic. Outside this central core is a peripheral region of large cells with deeply staining nuclei and dense cytoplasm, and the sheath of dermatogen with a few loosened layers of root-cap cells. There are no clearly defined plerome and periblem.

The first discernible differentiation of stelar tissue is the very early appearance of three protophloem strands abutting a well-defined uniseriate pericycle (fig. 2). These alternate with ridges of a triradiate central mass of larger parenchymatous cells, many of which later constitute the primary xylem. The cells of the protophloem are conspicuous because of their radial arrangement, the thinness of their cytoplasm, and the fact that their lateral walls are compressed by the adjacent meristematic cells. In longitudinal sections they appear to have elongated while adjacent cells continued to divide. The end walls are not tapering and there are no sieve plates. They retain their identity until after the protoxylem is differentiated. At the level of first appearance of the protophloem, the outermost layers of cortical cells become spherical, and intercellular spaces appear. The epidermis becomes clearly defined by the smaller size, long radial axis, and compactness of its cells.

In the region of most rapid elongation the metaphloem is differentiated, the sieve tubes developing conspicuous slime plugs very early. Companion cells ap-

pear at the same time but fibers are differentiated slightly later. Root hairs are not yet formed in this region but first appear where differentiation is most active. They are not confined to a definite zone but persist on all roots until the epidermis



FIGS. 1-4.*—Primary root of seedling: Fig. 1, longisection of root tip. Fig. 2, transection showing differentiation of protophloem and pericycle. Fig. 3, same at level of differentiation of protoxylem and endodermis. Fig. 4, same at level of completion of primary differentiation and formation of cambium.

* Symbols in all figures: *b*, tissue infected with bacteria; *ca*, cambium; *co*, cortex; *en*, endodermis; *ep*, epidermis; *f*, fiber; *mer*, meristem; *mx*, metaxylem; *pcl*, pericycle; *pd*, periderm; *ph*, phloem; *pi*, pith; *pph*, protophloem; *px*, protoxylem; *r*, ray; *rc*, root cap; *trc*, temporary root cap; *v*, vascular strand; *xyz*, secondary xylem.

is lost. Near the level of formation of the youngest root hairs the endodermis and protoxylem ridges are differentiated (fig. 3). The former consists of a single layer of the innermost cortical cells adjacent to the pericycle and having narrow Casparian bands. Each protoxylem ridge consists of one to four adjacent spiral or annular vessels, the outermost being smaller and next to the pericycle. The primary

root is triarch most commonly, but is not infrequently tetrarch, as shown by McMURRY and FISK (10) in their figures illustrating the condition of the tap root at the close of primary differentiation.

Whereas the outermost phloem cells differentiate as fibers first, sometimes giving the appearance of six bundles of fibers, others may soon form between them from parenchymatous cells adjacent to the pericycle, so that the whole phloem mass is frequently capped by fibers. Parenchyma, usually one or two layers of cells, separates the phloem and xylem. In this the cambium later arises by divisions tangential to the metaxylem (fig. 4).

Lignification of the metaxylem elements, which consist of cells with reticulate or simple-pitted walls, proceeds centripetally to include most of the central cells of the stele, though frequently some of these remain parenchymatous. Occurrence of an unusually large central metaxylem vessel is not uncommon.

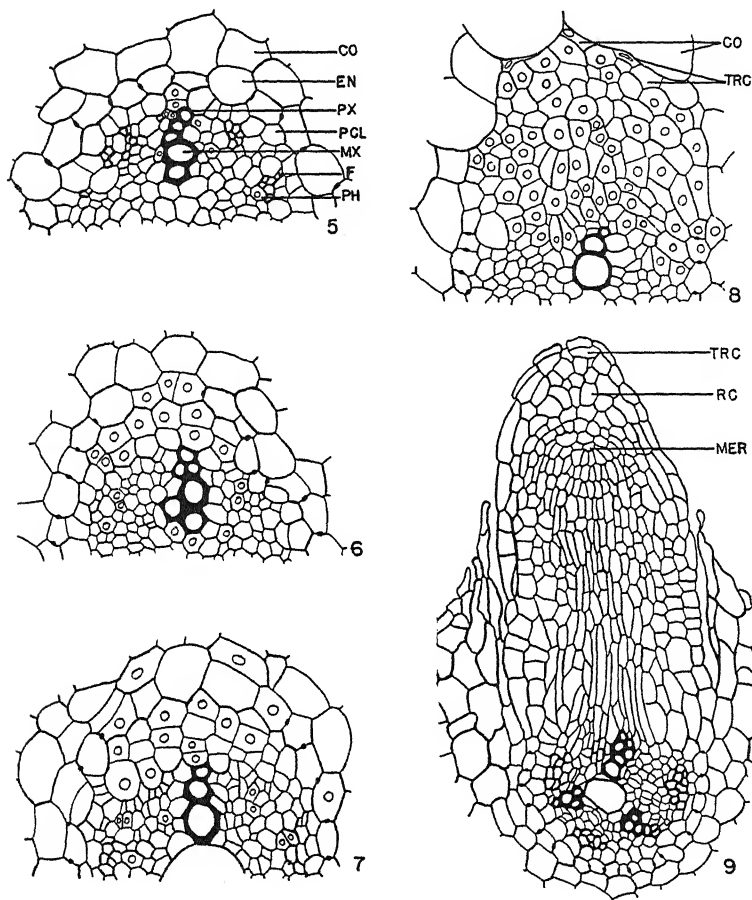
SECONDARY ROOTS

Before initiation of secondary growth and near the close of primary differentiation in the young tap root, lateral roots develop. These start growth through the periclinal divisions of a single pericyclic cell next to a protoxylem vessel, followed by similar divisions of a plate of adjacent pericyclic cells. Two successive periclinal divisions occur, followed by radial divisions and by stretching and enlargement of the overlying endodermal cells (figs. 5-7). As growth by further activity of pericyclic cells proceeds, the endodermal cells next to this new tissue likewise divide radially and tangentially, forming a temporary root cap at the tip, two or three cells in thickness. Most of the temporary cap cells are crushed against the cortical cells of the older root, are digested, or—after emergence of the root tip—are worn off or crushed by the soil. The cortical cells in turn are crushed, digested, or torn and pushed aside mechanically by the emerging root (figs. 8, 9).

The secondary roots develop a vascular system in a manner similar to that of the primary root. They are triarch usually, sometimes diarch, with a conspicuous endodermis.

During the third and fourth months, by which time a large amount of secondary tissue has developed near the crown of the plant, secondary roots arise in abundance on older roots and on the hypocotyl. Although the first node above the hypocotyl and the bud in the axil of the unifoliate leaf may become buried by soil and litter and remain alive during the winter of the first year, no adventitious roots were observed to arise above the first node. Adventitious and secondary roots, arising late in ontogeny, appear always opposite the primary rays, singly or in groups of two or three, emerging often close to the stump of an earlier rootlet which has died. The emergence of such roots is especially conspicuous during late autumn and winter months. Their formation begins in very early summer, when

the diameter of the primary root scarcely exceeds 1 mm., so that when the crown buds of the second year begin active growth, these roots have themselves branched and bear root nodules.



FIGS. 5-9.—Development of secondary root: Fig. 5, transection of portion of primary root showing initiation of secondary root by division of single pericycle cell. Fig. 6, same showing division tangentially of plate of pericycle cells and radial division of endodermal cell. Fig. 7, same showing second tangential division of pericycle cells and tangential division of endodermal cells. Fig. 8, longisection of young branch root showing crushing and digestion of cells of cortex of primary root and of temporary root cap. Fig. 9, transection of primary root showing emergence of secondary root.

The adventitious roots arise by meristematic activity of the cork cambium and underlying pericycle cells. The first vascular tissue of the young root differentiates through derivatives of the ray to that of the older root. The outer and more distal parts of the adventitious root are derived from the periderm, and its vascu-

lar system is later continued by differentiation of cells of its own histogen. As the adventitious root emerges, its base is surrounded by a glistening white mass of cells, formed by the cork cambium, which continues as a thin sheath over the root tip. The sheath is finally crushed, its cells disintegrating and leaving the young root free.

Subsequent development is not unlike that of other roots. The endodermis is more conspicuous than it is in the tap root. The adventitious roots are diarch or triarch most commonly, though tetrarch—and rarely pentarch—roots were found.

SECONDARY STRUCTURES

Cambial activity in the primary root begins when the seedling is 7–8 days old (10). During the third and fourth weeks of growth of the seedling and before the first trifoliate leaf has appeared, the cambium, at first limited to cells between the metaxylem and phloem, comes to involve the cells of the pericycle which are adjacent to the protoxylem. This starts the development of the primary rays and completes the cambium cylinder as secondary growth proceeds. At this stage numerous secondary roots have appeared and attained lengths of 1–2 mm., root nodules have developed, and the tap root is about 3 inches long. Root hairs cover the entire root system except the tips distal to the region of differentiation. The cork cambium has arisen in the endodermis.

During the second month several trifoliate leaves appear, and the hypocotyl begins to withdraw by contraction underground. The tap root attains a length usually exceeding 1 foot, and rootlets of the third order have appeared. Root hairs persist except in the upper part of the tap root where they have been lost with the rest of the epidermis after differentiation of the periderm.

By the end of the fourth month the tap root may have become several feet in length. Two or three of the lateral roots have developed into large branches, nearly equaling the tap root in diameter at the level of their divergence and in depth reached in the soil. Most of the lateral roots, however, remain short. At this time the largest diameter of the tap root about equals that of the hypocotyl and stem.

Throughout the late spring and summer of the first season a large body of vascular tissue develops, parenchyma remaining relatively inconspicuous. Transverse sections of the tap root show a compact mass of primary and secondary xylem and numerous narrow rays. The primary phloem persists, as yet uncrushed by secondary growth. The periderm is about twelve cells in thickness, and its outer suberized cells have sufficiently thick walls to show pits distinctly.

Cells of the vascular cambium are arranged in tiers as shown in longitudinal sections. They taper abruptly at the ends and are about $150\ \mu$ in length, those of the rays being more nearly isodiametric. The zone of meristem between the secondary xylem and phloem is three to five cells wide. Like the cambial cells, the sieve tubes

and companion cells of the secondary phloem are arranged in tiers, while gliding growth is characteristic of cells differentiating as fibers.

Fibers in the root are confined to the phloem and secondary xylem, not occurring in the primary xylem, cortex, pericycle, or periderm. Those in the xylem often show distinct pits. They vary greatly in length, those measured being 0.6–3 mm. in length at maturity.

The earliest formed secondary xylem consists of a few vessels with long slender pits and very large diameter, scattered within compact tissue composed mostly of fibers or small tracheids, and a few parenchymatous cells grouped mainly around the vessels. Many of the parenchymatous cells later develop thick pitted walls distinguishing the earlier secondary growth from that occurring later.

The portion developed after the second month has somewhat less compact xylem (figs. 10, 11). Cells of the cambium differentiate as new rays; the primary rays widen until they are, by the fourth month, six or more cells in thickness near the cambium. There is a tendency for cambium initials to differentiate as fibers for some time, and then for a period to differentiate as vessels and tracheids in close succession, so that alternating layers of fibers and conducting tissue resemble rings of growth. No striking contrast, however, distinguishes the growth of the first season from that of the second in most of the root. This is not true in the hypocotyl and uppermost part of the tap root. In these, during the winter months, more cambium cells remain parenchymatous and the vessels formed are small. In winter and early spring of the second season large vessels and numerous tracheids are again formed, giving a contrast which serves, for a while at least, to demark the two seasons of the vegetative cycle. JONES (6), working with alfalfa, reports layers of crushed phloem in addition, indicating successive years' growth. This was not seen in *Melilotus*.

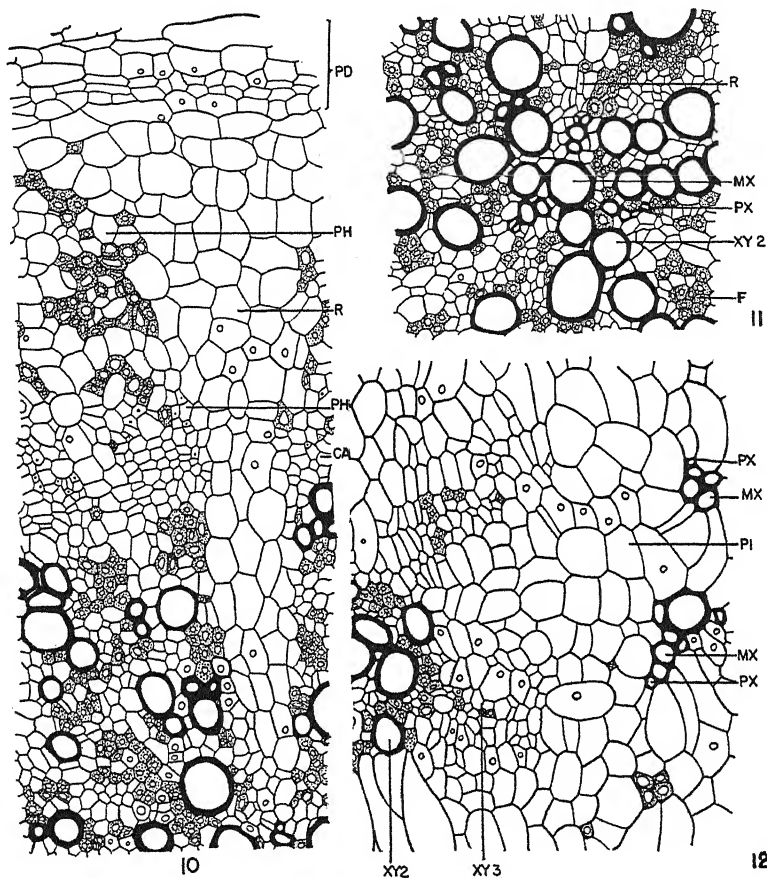
During the autumn and first season the most striking feature of secondary growth is the development of storage tissue. In this connection it will be necessary to consider the hypocotyl.

HYPOCOTYL AND DEVELOPMENT OF STORAGE TISSUE

In their study of the transition region in *Melilotus alba*, McMURRY and FISK (10) describe the change from the typical triarch condition of the primary root to diarchy or tetrarchy in the lower hypocotyl and the disappearance of the exarch ridge of xylem on the intercotyledonary axis at higher levels, so that there is no direct connection with the primary xylem of cotyledonary or plumular traces. They mention also the differentiation of a conspicuous pith in the hypocotyl of the seedling.

Secondary growth from a vascular cambium proceeds in the hypocotyl in a manner similar to that in the root. Likewise a cork cambium develops in the endo-

dermis continuous with that of the upper part of the tap root, and at the same time. It forms, however, a conspicuous layer of secondary tissue before loss of the primary cortex and epidermis, which occurs after the hypocotyl is completely underground. The hypocotyl is thenceforth not easily distinguishable externally

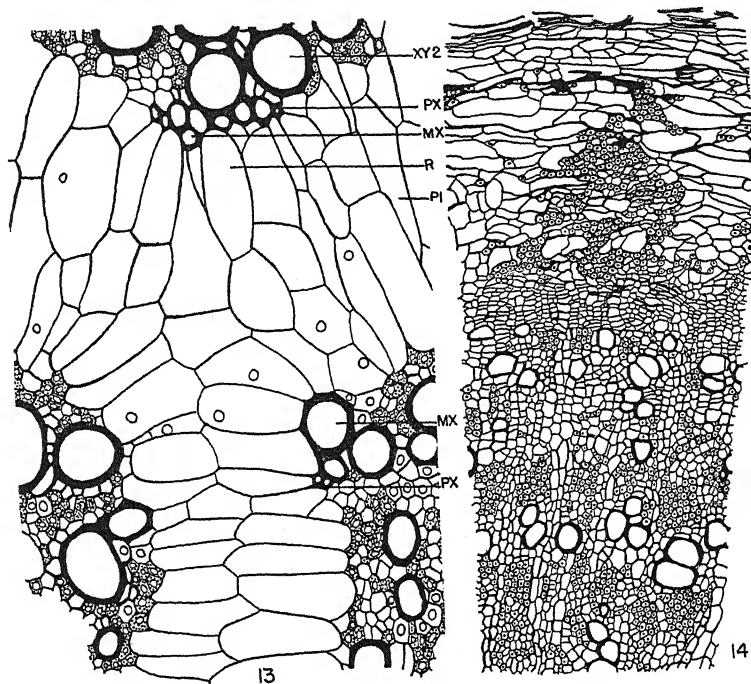


FIGS. 10-12.—Tap root of older plant: Fig. 10, transection of portion of tap root in June of first year showing secondary tissues. Fig. 11, same showing primary and secondary structures of central portion of stele. Fig. 12, central portion of tap root of second year showing tertiary tissue and separation of primary xylem elements by growth of parenchyma cells.

from the tap root. Internally the arrangement of the primary xylem makes such distinction possible until rapid growth of storage tissue involves the central elements of the stele.

In the tap root and hypocotyl, enlargement and radial divisions of cells in the pericycle and between the phloem groups become conspicuous in about the fourth

month, followed during the summer by general proliferation of the parenchyma, there and in the phloem itself. Phloem fibers become separated singly or in groups, and follow a sinuous course. During the same months all the rays are widened by multiplication and enlargement of their cells, but especially the central portions of the primary rays. Parenchyma associated with the metaxylem becomes active, the cells enlarge greatly, elongate tangentially, divide transversely, and then enlarge again. This results in separation of the metaxylem elements and in crushing



FIGS. 13, 14.—Tap root of plants of second year: Fig. 13, transection of central portion showing separation of elements of primary wood by growth of parenchyma. Fig. 14, transection of portion of dead root showing crushed phloem and thickened walls of parenchyma cells throughout most of area.

of the protoxylem (figs. 12, 13). The hypocotyl, upper part of the tap root, and larger secondary roots thus appear to possess a pith. Parenchymatous cells laid down close to the vessels of the secondary xylem, hitherto inconspicuous, enlarge and force the vessels and fiber groups apart. The activity of these cells in the central region of the hypocotyl is especially striking. The accumulation of starch in the storage tissue during the first season and its decrease during the second season have been described by WILLARD (15). Increase of the storage tissue continues in the second season but at a slower rate. A small amount of tertiary thickening may

occur in the pith, the cells maturing as fibers when any differentiation occurs (fig. 13).

What is apparently pith, which develops in the hypocotyl by proliferation of ray and xylem parenchyma, is to be distinguished in origin from the pith which first appears in the seedling associated with the primary tissues above the divergence of the two cotyledonary traces. In the old hypocotyl that pith appearing in the seedling is still conspicuous in the region of the cotyledonary node and is continuous with the pith of the stem rather than with that of the lower part of the hypocotyl, from which it is separated by a transverse plate of lignified tissue in which the parenchyma at the center of the stele becomes thick walled. This lignified tissue is confined to the cotyledonary node, suggesting that the hypocotyl in its structure is more nearly similar to the root than to the stem. It is readily distinguishable from the stem late in ontogeny as well as in the seedling.

Longitudinal sections through the old hypocotyl, at the time of flowering in the second season, show the primary vascular tissue and older vessels—and fibers of the secondary xylem in the central part of the stele—to be pushed laterally into sinuous folds by proliferating parenchyma, whereas the more recently formed vessels and fibers follow a straight course. This strongly suggests that proliferation of the parenchyma may have played an important role in the shortening and burial of the hypocotyl long before the cessation of secondary growth. JONES (6) suggests this explanation for shortening of the hypocotyl in alfalfa.

ROOT NODULE

The literature of the root nodule in the Leguminosae has recently been reviewed by FRED, BALDWIN, and MCCOY (3) and WILSON (16).

On the primary root of field-grown seedlings of *Melilotus* subjected only to chance infection by root nodule bacteria, nodules appear externally 7 days after germination of the seeds. At this age the unifoliate leaf has appeared but has not yet expanded. Even earlier than this nodules can be seen within the cortex of seedlings which have been cleared in chloral hydrate or in xylol. They become conspicuously enlarged and are more abundant by the tenth day, at which time the unifoliate leaf of the seedling is fully expanded. The fact that few nodules are formed before the appearance of the first true leaf in *Medicago* has been noted and regarded as significant by THORNTON (12). Secondary roots, after the appearance of root hairs, may also develop nodules.

Since divisions of the cells in the very young nodule are in many planes, a globular mass of tissue develops before the emergence of the nodule beyond the surface of the root. This enlarges and, after emergence, develops a cylindrical shape and becomes pink in color.

Transverse sections of mature nodules show the usual central mass of paren-

chymatous cells, most of which contain bacteria. A sheath of cells, containing starch in abundance, envelopes the central mass except at its distal end where a terminal meristem continually forms additional nodular tissue. The mature nodule has an outer cortical region of uninfected cells, beneath which—and outside the starch sheath—the nodule is traversed by two vascular strands differentiated back to the stele of the root opposite one, or occasionally two, of the protoxylem ridges.

The nodules of *Melilotus alba* are annual. Before the end of their growth they may become branched, the two vascular strands within the nodule likewise being branched repeatedly. At the end of the season the most central tissue of the nodule, containing the bacteria, disintegrate, leaving for a while a soft brown shell of cortical and vascular tissues. Eventually this too is lost and the bacteria within are returned to the soil.

A description of the ontogeny of the nodule is not undertaken here. Recent investigations of the possible role of growth substances, of metabolic products of the bacteria, and of growth-stimulating materials of the soil in the infection of roots and in the initiation and development of root nodules in the Leguminosae, and speculations in regard to these, have raised new questions and reopened old ones. Many of these have been pointed out by WILSON (16). Likewise cytological studies in which the tetraploid nature of the infected cells of the nodule has been described (17, 18, 19) seem to indicate that the whole matter of the ontogeny of the root nodule merits reinvestigation.

DEGENERATION AND DEATH OF ROOT

The period of flowering extends for about 6 weeks early in the summer of the second season. During this time the growth of the tops greatly exceeds that of the roots, and the weight of the root is reduced by loss of reserve foods (15). After subsequent formation of the last seeds the plant dies, usually in August or September. The root dies first.

Before death of the root, signs of degeneration appear. The older phloem is crushed by secondary growth, causing collapse of the parenchyma, sieve tubes, and some of the fibers. At the same time the interfascicular parenchyma collapses so that a sheath of dead tissue is formed beneath the periderm (fig. 14). Activity of the cambium ceases, as shown by the maturity of all the tissues most recently formed from it, so that the zone of undifferentiated cells between the xylem and phloem is narrowed down to a single layer of cells constituting the cambium. A marked increase in the number of fibers differentiated in the central part of the stele and general thickening of the walls of parenchymatous cells occur throughout the root, with the exception of the isolated periderm. Slight thickening of the walls of the youngest phloem and of the cambium cells takes place, occurring first in the rays, beginning near the center of the root, but becoming most marked in

the second year's growth. These changes occur first in the younger roots, progress to the older ones, and finally involve the tap root and hypocotyl.

The earliest indication of death of the tissues is shriveling of the cytoplasm. In sections of larger roots made before they seem to be dead, brown or black discolored areas occur. These mark the bases of dead lateral roots whose phloem gives the staining reactions characteristic of necrotic tissue. Through these lateral roots saprophytic fungi soon invade the larger roots, attacking the phloem, and then—following the rays inward—destroy the pith and finally plug the vessels by their growth. The entire life of the biennial plant occupies about 19 months from the time of germination of the seed.

Summary

1. The ontogeny of the primary root of *Melilotus alba* is of the fourth angiospermous type of JANCZEWSKI, in which there is a general meristem from which arises the cortex, stele, and central portion of the root cap. The meristem is extended laterally to form the outer parts of the root cap, and at higher levels a dermatogen.

2. Protophloem is the earliest stelar tissue differentiated.

3. Secondary roots arise by cell divisions in the pericycle opposite the protoxylem ridges. The endodermis forms a temporary root cap, which is ultimately crushed or digested, as are also cells of the cortex and epidermis of the primary root by the emerging root. The vascular system is developed in a manner similar to the primary root.

4. Lateral roots develop in the periderm and primary rays of old roots and of the hypocotyl. Cells of the periderm, by radial and tangential divisions, give rise to a temporary sheath over the young root, which is later ruptured. Subsequent development is similar to that of the secondary roots.

5. Secondary tissues develop from a cork cambium which arises in the endodermis, and from a vascular cambium which arises in the seedling between the xylem and phloem. A small amount of tertiary tissue may develop in the pithlike mass of the stele of the tap root late in ontogeny, maturing chiefly as fibers.

6. Parenchyma throughout most of the older roots, especially in the primary rays, becomes active late in the season, separating by its proliferation groups of vessels, fibers, and sieve tubes, crushing the protoxylem, and forming a pithlike mass. It serves as storage tissue for a large reserve of starch.

7. Withdrawal of the hypocotyl underground accompanies proliferation of the parenchymatous tissue and increase in the diameter of the hypocotyl and tap root late in the first season.

8. The hypocotyl has a pith above the level of divergence of cotyledonary traces continuous with that of the stem and present in the seedling. Late in on-

togeny it develops also a pithlike mass by proliferation of parenchyma in central parts of the stele, which is continuous with that of the root and homologous with it.

9. The root nodule originates in young roots near the close of primary differentiation. Two vascular strands are differentiated which are connected with the stele of the root opposite the nearest protoxylem ridges. An apical meristem adds cells to a central mass of infected and starch-bearing tissues and to a cortex which invests the entire nodule.

10. Root nodules are annual. New ones develop in succession on young roots.

11. Secondary growth continues in the second year through the period of flowering, but at a slower rate. Degeneration occurs after seeds are formed. The older phloem is crushed, the periderm becomes isolated by a layer of dead tissue beneath, most parenchymatous tissue throughout the root develops thick walls, and the cambium ceases to form new tissue. Increase of fibers in the center of the stele is marked.

12. The roots die before the tops; small roots die before the large ones. The tissues finally become disintegrated by the action of saprophytic fungi. Nineteen months is the length of life of the plant from the time of germination of the seed.

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LITERATURE CITED

1. COOPER, D. C., Macrosporogenesis and embryology in *Melilotus*. BOT. GAZ. 95:143-155. 1933.
2. ELDERS, A. T., Some pollination and cytological studies of *Melilotus alba*. Sci. Agr. 6:360-365. 1926.
3. FRED, E. B., BALDWIN, I. L., and MCCOY, ELIZABETH, Root nodule bacteria and leguminous plants. Univ. Wisconsin Studies Sci. 5. 1932.
4. GUIGNARD, M. L., Recherches d'embryogenie vegetale comparés: Legumineuses. Ann. Sci. Nat. Bot. Ser. VI. 12:5-176. 1880-1881.
5. JANCZEWSKI, E., Recherches sur le développement des racelles dans les phanérogames. Ann. Sci. Nat. Bot. Ser. V. 20:208-233. 1874.
6. JONES, F. R., Winter injury of alfalfa. Jour. Agr. Res. 37:189-211. 1928.
7. LLOYD, W. A., Sweet clover (*Melilotus*), a field survey of its distribution, soil adaptation, habits and agricultural value. Ohio Agr. Exp. Sta. Bull. 244. 1912.
8. LOVE, H. H., and LEIGHTY, C. E., Germination of seed as affected by sulphuric acid treatment. Cornell Agr. Exp. Sta. Bull. 312. 1912.
9. MARTIN, J. N., The relative growth rates and interdependence of tops and roots of the biennial sweet clover, *Melilotus alba* Desv. Amer. Jour. Bot. 21:140-159. 1934.
10. MCMURRY, E. B., and FISK, EMMA L., Vascular anatomy of the seedling of *Melilotus alba*. BOT. GAZ. 98:121-134. 1936.

11. SNIDER, H. J., and HEIN, M. A., Nitrogen and dry matter content of sweet clover tops and roots at various stages of growth. Jour. Amer. Soc. Agron. 18:273-280. 1926.
12. THORNTON, H. G., The influence of the host plant in inducing parasitism in lucerne and clover nodules. Proc. Roy. Soc. London Ser. B. 106:110-122. 1930.
13. WATT, J. R., Simultaneous development of the seed coat and embryo in the seeds of sweet clover. Proc. Iowa Acad. Sci. 37:117-123. 1930.
14. WEAVER, J. R., Root development of field crops. New York. 1926.
15. WILLARD, C. J., An experimental study of sweet clover. Ohio Agr. Exp. Sta. Bull. 405. 1926.
16. WILSON, P. W., The biochemistry of symbiotic nitrogen fixation. Univ. Wisconsin Press. 1940.
17. WIPF, LOUISE, Chromosome numbers in root nodules and root tips of certain Leguminosae. BOT. GAZ. 101:51-67. 1939.
18. WIPF, LOUISE, and COOPER, D. C., Chromosome numbers in nodules and roots of red clover, common vetch, and garden peas. Nat. Acad. Sci. Proc. 24:87-91. 1938.
19. ———, Somatic doubling of chromosomes and nodular infection in certain Leguminosae. Amer. Jour. Bot. 27:821-838. 1940.
20. YOUNG, W. S., The embryology of *Melilotus alba*. Proc. Indiana Acad. Sci. 1905:131-141. 1905.

EFFECT OF LENGTH OF INDUCTION PERIOD ON FLORAL DEVELOPMENT OF *XANTHIUM PENNSYLVANICUM*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 528

FRANCES LLOYD NAYLOR

(WITH FOURTEEN FIGURES)

Introduction

Xanthium pennsylvanicum has been classified as a short-day plant. It tends to produce only vegetative growth on 24-hour cycles consisting of more than 16 hours of light and less than 8 hours of darkness and tends to flower when the photoperiods are shorter than $15\frac{1}{2}$ hours with accompanying dark periods of more than $8\frac{1}{2}$ hours. If exposed to a few cyclic alternations of light and darkness favorable for flowering, a plant may subsequently flower even though transferred to cycles which with continuous treatment would tend to result in purely vegetative activity. HAMNER (3) has termed these cyclic alternations of light and darkness which tend to induce flowering "photoinductive cycles." With *Xanthium* the so-called short day would constitute a photoinductive cycle; but, as he has shown, other cycles may induce floral initiation even though such cycles could not properly be considered under the term short day. In *Xanthium* a photoinductive cycle contains a specific minimum photoperiod of definite minimum intensity followed by a continuous dark period of more than 9 hours (3).

NEIDLE (5) and HAMNER (3) have found that the rate and nature of the development of the inflorescences of *Xanthium* are affected by the environmental conditions subsequent to the induction period. The former studied the effects of nitrogen supply and the latter primarily the effects of various conditions of light during the postinductive period.

It has been shown (4) in *Xanthium* that one photoinductive cycle may result in subsequent initiation of floral primordia. Under such conditions the rate of development of these primordia into mature flowers and fruits may be very slow. Since treatment with more than one photoinductive cycle seems to affect the rate of floral development, and since it has been shown with Biloxi soybean (1) also that the rate of floral development and the number of flowers initiated are affected by the number of photoinductive cycles to which the plants are exposed, an anatomical study of the floral development in plants which had been exposed to various numbers of photoinductive cycles was made.

Procedure

The experiments reported here were conducted in the summer of 1939. *Xanthium* plants were planted on August 1 in sandy garden soil in clay pots and grown in the greenhouse. During the first 25 days all plants were grown under conditions of long photoperiod, being exposed to supplementary illumination from dusk until midnight. This illumination was obtained from 200-watt Mazda filament lamps and supplied about 80 foot candles at the surface of the leaves. Subsequent to the twenty-fifth day the plants were exposed to the various experimental treatments. In these treatments continuous illumination was obtained by burning the lamps from dusk until sunrise. Photoinductive cycles (short photoperiods) were produced by placing the plants on trucks and moving them into dark compartments at 4:00 P.M., where they remained until 8:00 A.M. the following morning. From then until 4:00 P.M. the trucks stood in the lighted greenhouse.

On the twenty-fifth day the terminal buds of some of the plants were removed and preserved for subsequent examination. All such buds proved to be strictly vegetative, and the assumption is made that all comparable plants were vegetative at this time. As a further check, certain of the plants were continued for 4 more days under long-photoperiod conditions on the greenhouse bench, and samples were preserved and examined histologically. These were also strictly vegetative. The remaining plants were started on the various experimental treatments after the twenty-fifth day (August 25). One lot of 320 plants was exposed continuously to photoinductive cycles, and terminal buds of ten plants were collected each day for 32 days and preserved for histological examination. These plants of lot 1 were used as a basis of comparison. The second lot, containing 50 plants, was transferred to continuous illumination; the third lot of 100 plants was exposed to one photoinductive cycle; a fourth lot of 100 plants, to four photoinductive cycles; and a fifth lot of 100 plants, to eight photoinductive cycles. Lots 3-5, inclusive, were placed under continuous illumination subsequent to their photoinductive treatment. The terminal nodes of ten plants of each of lots 2, 3, 4, and 5 were collected at weekly intervals for 10 weeks and preserved for histological examination, the first collection being made September 2.

The specimens were preserved in formalin-acetic-alcohol and handled according to the butyl-alcohol paraffin method. Serial longitudinal and transverse sections were cut at 15 μ and stained with safranin, gentian violet, and orange G.

Observations

CONTROL PLANTS

The terminal buds of the plants collected on August 25 were strictly vegetative, as were those from the same bench collected on August 29. The plants of lot 2, which were transferred on August 25 to continuous illumination, were all typically

vegetative at each sampling (fig. 1). From these results it is assumed that all plants were vegetative on August 25, and that plants of groups 3, 4, and 5 which developed floral primordia and flowers while growing in continuous illumination did so because of previous photoinductive treatment.

CONTINUOUS PHOTOINDUCTIVE TREATMENT (LOT 1)

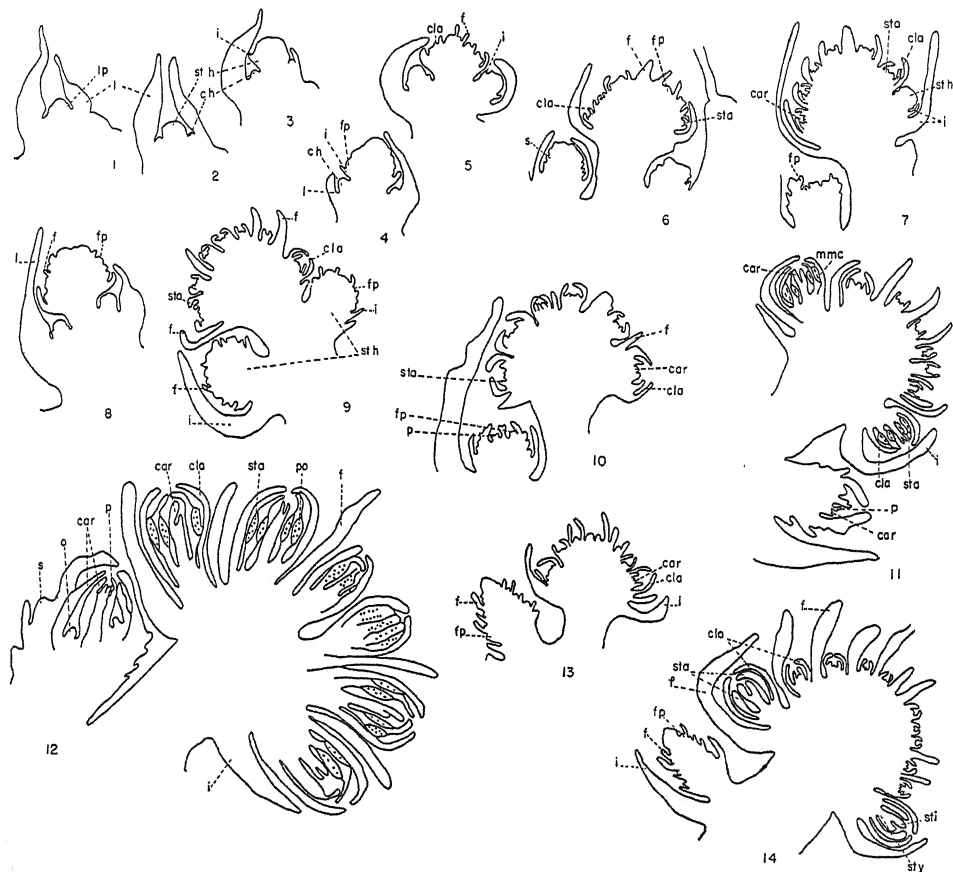
The terminal buds of plants collected on the first and second days after the beginning of photoinductive treatments showed no noticeable change from the vegetative condition, but on the third day the terminal growing point was broad, smooth, somewhat elevated, and hemispherical (fig. 2). This was the primordium of the terminal staminate inflorescence. By the fourth day the primordia of the involucre bracts appeared at the base of the terminal staminate inflorescence. In the axils of the young leaves, just below the staminate inflorescence, there appeared the primordia of the carpellate inflorescences (fig. 3). By the fifth day the first flower primordia of the staminate inflorescence were noticeable and the carpellate inflorescence primordia had enlarged to about 0.15 mm. (fig. 4). From this point on the development of the staminate and carpellate inflorescences is described separately.

STAMINATE INFLORESCENCE.—The flower primordia and the primordia of the floral bracts developed in spiral acropetalous succession in the staminate inflorescence and were numerous by the sixth day (fig. 5). The primordia of the central flowers had flattened out and the primordia of the marginal flowers had not only flattened but the initials of the corolla were present at their margins. By the seventh (fig. 6) and eighth days (fig. 7) the inflorescence was about 1.3 mm. in diameter, and the slender peduncle by which the terminal staminate inflorescence is raised above the carpellate inflorescences had elongated, its length being about equal to the diameter of the staminate inflorescence. The primordia of the corolla were present in the central flowers, and in the marginal flowers the corolla primordia and also the stamen primordia were present, the latter arising adaxial to the corolla. Each flower was in the axil of a bract.

At this time one or two small staminate inflorescences (about 0.26 mm. in diameter) were noted within the terminal staminate inflorescence, each small one appearing in the axil of an involucre bract of the terminal inflorescence. Flower and floral bract primordia were present in the small inflorescences.

On the ninth day (fig. 10) the terminal staminate inflorescence was 1.8 mm. in diameter. Each flower had a marginal sympetalous corolla, a column composed of five or six undiverged stamens, and the primordia of two undiverged carpels in the center. In some flowers a cleft had appeared at the apex of the carpel primordia. The calyx was absent. By the thirteenth day (fig. 12) each stamen was differentiated into filament and anther. Pollen was shed about the eighteenth day after the

beginning of induction. The rudimentary pistil of each staminate flower consisted of a style with a bifid stigma. The period during which pollen was shed extended beyond the time of shedding from the terminal inflorescence, since the flowers of



FIGS. 1-14.—Terminal region of *Xanthium* represented in each figure. Fig. 1, vegetative plant. Fig. 2, third-day stage of plant which had received continuous photoinductive treatment (lot 1). Fig. 3, fourth day of same. Fig. 4, fifth day. Fig. 5, sixth day. Fig. 6, seventh day. Fig. 7, eighth day. Fig. 8, eighth-day stage of plant which had received one photoinductive cycle followed by continuous illumination (lot 3). Fig. 9, eighth-day stage of plant which had received four photoinductive cycles. Fig. 10, ninth-day stage of lot 1. Fig. 11, eleventh-day stage of lot 1. Fig. 12, thirteenth day of same. Fig. 13, thirty-sixth-day stage of lot 3. Fig. 14, sixty-fourth-day stage of lot 3. (car, carpel; cla, corolla; ch, carpellate inflorescence; f, floral bracts; fp, flower primordium; i, involucre bract; l, leaf; lp, leaf primordium; mmc, microspore mother cell; o, ovule; po, pollen; s, spine; sta, stamens; sti, stigma; sth, staminate inflorescence; p, perianth; sty, style.)

the small inflorescences in the axils of the involucre bracts developed more slowly than did the flowers of the terminal inflorescence itself.

CARPELLATE INFLORESCENCE.—The primordia of the involucre bracts were

present at the base of the primordium of the topmost carpellate inflorescence by the sixth day (fig. 5). By the seventh day the inflorescence was 0.6 mm. in diameter and many protuberances had appeared on the surface. The lower ones, about fifteen in all, were the primordia of the involucre bracts; the upper ones were the primordia of the spines. The receptacle of the carpellate inflorescence was flattened at the apex (fig. 6). The margins of the receptacle, and the tissue at the exact center between the margins, became slightly elevated. Continued growth of these regions resulted in the formation of two depressions, which became wide at the bottom and narrow above. On one side the margin grew more rapidly and thus one depression became deeper than the other.

By the eighth day flower primordia appeared simultaneously in the bottom of each depression. The flower in the lower depression developed somewhat more rapidly and became larger than the other. The central tissue, which was a partition between the two depressions, developed a cleft in its upper surface (fig. 7). By the ninth day (fig. 10) the primordium of the perianth was evident at the margin of each flower primordium. By the eleventh day the two carpels had appeared in the center of the flower (fig. 11). The upward growth of the carpels formed the single cavity of the ovary. The carpels grew much more rapidly than did the perianth, and by the thirteenth day (fig. 12) the perianth appeared as a small flange on the outer margin of the carpels. The carpels continued elongation and formed a long style and a bifid stigma. About the twelfth day an ovule began developing in each ovary.

All flower parts were present by the thirteenth day, and further development consisted of increase in length and breadth. The megagametophyte was mature by the seventeenth day. The margin and central tissue of the receptacle grew far above the flowers. One part of the central tissue, with half the margin, formed the beak above one flower. The other half of the central tissue and the other half of the margin formed the beak above the second flower.

The carpellate inflorescences in the axils of the third and fourth leaves below the terminal staminate inflorescence developed more rapidly and became larger than did the uppermost carpellate inflorescences. For example, on the eighth day the flower primordia were present in the depressions of the uppermost inflorescences, but in the lower inflorescences the perianth and carpel primordia were already differentiated. At the end of a month the seed of the uppermost bur was about 3.5 mm. long and the embryo about 1.5 mm. long. The lower bur contained a seed 6.5 mm. long and an embryo 2.5 mm. long.

FARR (2) has stated that the bur is a modified capitulum and that it differs from the typical capitulum of the Compositae only in having two depressions in the receptacle. He believes that the spines are specialized bracts and the beaks are portions of the receptacle.

VARIOUS INDUCTIVE TREATMENTS (LOTS 3-5)

1. ONE PHOTOINDUCTIVE CYCLE.—The inflorescences and flowers of these plants were greatly delayed in development as compared with those which received continuous photoinductive treatment (lot 1). By the eighth day (fig. 8) after induction the staminate inflorescence showed the primordia of the flowers and leaf bracts, but no flower parts had differentiated. The primordia of the involucre bracts of the inflorescences in the axils of the young leaves were just visible. This stage was shown on the fifth day by the plants of lot 1. By the fifteenth day the marginal flower primordia of the terminal staminate inflorescence showed flattened apices. The floral bracts were clearly distinguishable from the flower primordia, a stage comparable with that of the plants of lot 1 on the sixth day. The axillary inflorescences showed little advance in development, the inflorescences being somewhat cone-shaped, the involucre bracts differentiated, but the type of inflorescence not yet distinguishable.

By the twenty-second day the marginal flowers of the terminal staminate inflorescence had developed the primordium of the corolla and a few of the central flowers had flattened apices, a stage comparable with the plants of lot 1 on the seventh day. The axillary inflorescences showed no change in development. By the twenty-ninth day the marginal flowers of the terminal staminate inflorescence resembled those of lot 1 on the eighth day, having the corolla, stamen, and carpel primordia established; but the most central flowers were just beginning to show corolla primordia. There was a greater difference in the size and stage of development between the outer and inner flowers of this inflorescence than in those of lot 1. This difference became increasingly marked as the plants grew older. The peduncle by which the terminal staminate inflorescence is raised above the topmost leaves was longer than in the plants of lot 1.

In the terminal staminate inflorescence there were one or two axes, each appearing in the axil of an involucre bract of the terminal staminate inflorescence. Each axis was composed of one small terminal and two small lateral staminate inflorescences, rather than of a single staminate inflorescence as in lot 1. These small inflorescences had flower primordia and bracts but no flower parts differentiated. Unlike the small staminate inflorescences of lot 1, these were elongated. The internodes of each small inflorescence had continued longitudinal growth and the individual flowers developed one above the other on the axis in the axils of the floral bracts. By the thirty-sixth day the small staminate inflorescences had increased in size, and by the forty-third day the floral bracts were very long. It seems probable that these floral bracts may become the leafy organs often noted on staminate inflorescences of *Xanthium* plants which have received a few induction periods and then been grown on long photoperiod for several months (5).

An individual carpellate inflorescence or an axis consisting of three carpellate

inflorescences usually appeared in the axils of the first, second, third, and fourth leaves below the terminal staminate inflorescence in the plants of lot 1. In some of the plants which received only one photoinductive cycle (lot 3) a lateral axis consisting of three small staminate inflorescences appeared in the axil of the topmost leaf. These inflorescences were elongated rather than round. In the terminal inflorescence of this lateral axis flower primordia and floral bracts were present. In the axils of other upper leaves there were carpellate inflorescences. In the axils of leaves below these the primordia were so slightly developed that their type could not be determined.

By the thirty-sixth day all the flower parts had formed in the marginal flowers in the terminal staminate inflorescence, the largest staminate inflorescence of the plant. The central flowers, however, were still in the primordial stage (fig. 13). By the sixty-fourth day nearly all the central flowers of the terminal staminate inflorescence had formed corolla and stamen primordia (fig. 14). Carpellate inflorescences in which the depressions were beginning to form were found in the axils of some of the upper leaves of the plant. The inflorescences did not develop further before the end of the experimental period.

2. FOUR PHOTOINDUCTIVE CYCLES.—These plants had received four photoinductive cycles (four short photoperiods) and 4 days of continuous illumination when the first samples were taken at the end of the eighth day (fig. 9). In the terminal staminate inflorescence the individual flowers were nearly as well developed as were those of lot 1. The one or two small staminate inflorescences which appeared in the axils of involucre bracts of the terminal staminate inflorescence were about three times as large as those on the plants of lot 1, being about 0.8 mm. in diameter. Their flower and bract primordia could be distinguished, but no flower parts had been differentiated. The staminate inflorescences were round, not elongated like the small ones on the plants which had received one cycle of induction. Subsequent development was somewhat delayed, a stage comparable with that of lot 1 on the eleventh day being reached by the fifteenth day. The first pollen was shed about 3 weeks after the shedding by plants of lot 1. The megagametophytes were mature by the thirty-sixth day. The inflorescences in the axils of the involucre bracts formed their flower parts later than did the terminal inflorescence itself.

3. EIGHT PHOTOINDUCTIVE CYCLES.—At the time of the first collection of lot 5, 8 days after the beginning of the photoinductive treatments, these plants had not yet been moved to continuous illumination. They were in the same stage of development as were the plants of lot 1 on the eighth day. Samples from later collections, taken after these plants had been moved to continuous illumination, showed that development (as compared with lot 1) was only slightly delayed. By the twenty-second day the megagametophytes were mature. Pollen was shed about 5 days after the shedding by the plants of lot 1.

The number of staminate and carpellate inflorescences in the upper five nodes of

plants which had received one, four, and eight photoinductive cycles was compared with the number on the plants under continuous photoinductive treatment (lot 1). Stages from each group, comparable with the thirteenth day of the latter (the eighty-fourth-day stage of the plants induced one day, etc.), were selected because at this stage the character of the inflorescences could best be determined.

On the plants of lot 1 there were present a terminal staminate inflorescence and also two small staminate inflorescences, each in the axil of an involucre bract of the terminal inflorescence. The inflorescences in the axils of the four topmost leaves were carpellate. In some plants the axis in the axil of the fifth leaf below the terminal staminate inflorescence bore lateral carpellate inflorescences and terminated in a staminate inflorescence. There were three or four staminate and twelve or thirteen carpellate inflorescences per plant at the upper five nodes.

On the plants which received one photoinductive cycle the main axis terminated in a staminate inflorescence. Two lateral axes, each in the axil of an involucre bract of the terminal inflorescence, were each composed of three small staminate inflorescences. In the axil of the topmost leaf of some plants there were three staminate inflorescences; those in the axils of two other upper leaves were carpellate. In many plants the axes in the axils of the fourth and fifth leaves below the terminal staminate inflorescence were so slightly differentiated that it was impossible to tell whether they were vegetative or floral. There were about ten staminate and six carpellate inflorescences in the upper five nodes.

The plants which received four photoinductive cycles and the ones which received eight had about the same number of staminate and carpellate inflorescences as had the plants which received continuous photoinductive cycles (lot 1).

Summary

1. Histological examinations were made of the developing carpellate and staminate inflorescences of *Xanthium pennsylvanicum*. The plants were exposed after the twenty-fifth day of vegetative growth to (a) continuous photoinductive cycles, (b) one photoinductive cycle, (c) four photoinductive cycles, (d) eight photoinductive cycles. The study included those inflorescences which developed from the terminal bud and the buds in the axils of the five uppermost leaves.

2. Development of both carpellate and staminate inflorescences in the plants exposed continuously to photoinductive cycles was more rapid than that in any other group. All flower parts were present by the thirteenth day, and at the end of the month the seeds were almost mature.

3. Development of inflorescences in the plants which received only one photoinductive cycle was much slower than that of plants which received continuous photoinductive treatment. The flower parts of the former did not appear in all the flowers of the terminal staminate inflorescence until the sixty-fourth day after induction, rather than by the thirteenth day as in the latter. The former also dif-

ferred from the latter in that there was a greater difference in the size and stage of development between the outer and inner flowers of the inflorescence.

4. The terminal staminate inflorescence of the plants receiving one photoinductive cycle, as well as of the plants exposed continuously to photoinductive treatment, contained one or two axes, each appearing in the axil of an involucre bract of the terminal inflorescence. Each axis of the former was composed of three staminate inflorescences, rather than of a single one as in the latter. The small staminate inflorescences of the former were much more elongated than those of the latter.

5. In the axil of the uppermost leaf a lateral axis consisting of three staminate inflorescences appeared in some of the plants exposed to one photoinductive cycle, while in those exposed continuously to photoinductive treatment an individual carpellate inflorescence or an axis consisting of three usually occupied this position. The staminate inflorescences of the former were elongated rather than round.

6. In the plants exposed to one photoinductive cycle there developed approximately ten staminate and six carpellate inflorescences, while in the plants exposed continuously to photoinductive cycles there was an average of about four staminate and twelve or thirteen carpellate inflorescences per plant. Thus, increasing the number of induction treatments seemed to stimulate the production of carpellate more than of staminate inflorescences.

7. The plants which received four photoinductive cycles exhibited, in the axils of involucre bracts of the terminal staminate inflorescence, one or two staminate inflorescences three times as large as those occupying this position in plants exposed continuously to photoinductive treatment.

8. The development of the inflorescences on those plants which received four or eight photoinductive cycles resembled more closely the development in plants continuously exposed to such treatment than that in plants exposed to one photoinductive cycle. The rate of development was intermediate between the two.

The writer expresses her appreciation of the helpful suggestions given by DR. KARL C. HAMNER during the course of these experiments.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans. BOT. GAZ. 99:825-839. 1938.
2. FARR, C. H., The origin of the inflorescences of *Xanthium*. BOT. GAZ. 59:136-148. 1915.
3. HAMNER, K. C., Interrelation of light and darkness in photoperiodic induction. BOT. GAZ. 101:658-687. 1940.
4. HAMNER, K. C., and BONNER, J., Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388-431. 1938.
5. NEDDLE, EDITH K., Nitrogen nutrition in relation to photoperiodism in *Xanthium pennsylvanicum*. BOT. GAZ. 100:607-618. 1939.

COMBINED EFFECTS OF POTASSIUM SUPPLY AND GROWTH SUBSTANCES ON PLANT DEVELOPMENT¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 529

HORTON M. LAUDE

(WITH SIX FIGURES)

Introduction

The reported dry-weight determinations of plants treated with growth-regulating substances have indicated both losses and gains associated with such treatment. GRACE (4), HWANG and PEARSE (6), MCROSTIE, HOPKINS, and GRACE (8), MITCHELL and HAMNER (9), and THIMANN and LANE (17) have all reported increased dry weight of various plants owing to such treatments. PEARSE (12, 13) found the dry weight of leaf and root decreased while that of stem and petiole increased in tomatoes, yet the total dry weight did not increase. TEMPLEMAN (16), using white mustard and barley, reported only significant depressing effects from growth substance treatment and no stimulation of top or root weights.

The combined effects of variation in nutrient supply and growth-regulating substances have been studied in a few instances. Nutrition as it affects the production of growth substance within the plant has been investigated by AVERY, BURKHOLDER, and CREIGHTON (1, 2) and by SKOOG (15). Nutritional levels and growth-substance treatments in relation to root development and carbohydrate and nitrogen distribution within the plant have been reported by SMITH, NASH, and DAVIS (14). EATON (3) suggested the ability of externally-applied indoleacetic acid partially to replace boron as an essential element. SKOOG (15) noted that applied indoleacetic acid may enhance the utilization of zinc.

The investigation here reported deals with the effects—at different levels of potassium—of growth substance supplied in the nutrient medium to sand cultures of kidney bean.

Material and methods

Red kidney bean, *Phaseolus vulgaris*, was grown in white quartz sand in 4-inch glazed pots. Before planting the seed the sand was thoroughly wet with distilled water and given one application of minus potassium nutrient. This first addition of nutrient was necessary to insure uniform and vigorous plants soon after emer-

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gence; without it the plants did not properly expand their first foliage leaves. Four selected seeds were planted at uniform depth in each pot. As soon as the heart-shaped leaves were expanded, the plants were thinned to two per pot. Seed coats and ungerminated seeds were removed.

Five nutrient solutions, designated as minus K, $1/18$ K, $3/9$ K, $9/9$ K, and $18/9$ K, were prepared in the following manner. The amount of 0.5M stock solution of the reagent quality salts used for every liter of nutrient solution is listed. Distilled water was employed in all solutions.

The minus K nutrient solution consisted of 12 cc. of 0.5M $\text{Ca}(\text{NO}_3)_2$, 9 cc. of 0.5M MgSO_4 , and 9 cc. of 0.5M NaH_2PO_4 per liter. The $1/18$ K nutrient solution contained 12 cc. of 0.5M $\text{Ca}(\text{NO}_3)_2$, 9 cc. of 0.5M MgSO_4 , 0.5 cc. of 0.5M KH_2PO_4 , and 8.5 cc. of 0.5M NaH_2PO_4 per liter. The $3/9$ K nutrient solution consisted of 12 cc. of 0.5M $\text{Ca}(\text{NO}_3)_2$, 9 cc. of 0.5M MgSO_4 , 3 cc. of 0.5M KH_2PO_4 , and 6 cc. of 0.5M NaH_2PO_4 per liter. The $9/9$ K nutrient solution employed 12 cc. of 0.5M $\text{Ca}(\text{NO}_3)_2$, 9 cc. of 0.5M MgSO_4 , and 9 cc. of 0.5M KH_2PO_4 per liter. The $18/9$ K nutrient solution consisted of 12 cc. of 0.5M $\text{Ca}(\text{NO}_3)_2$, 9 cc. of 0.5M MgSO_4 , 9 cc. of 0.5M KH_2PO_4 , and 9 cc. of 0.5M KCl per liter. A somewhat higher osmotic concentration resulted in the $18/9$ K level owing to the addition of KCl to the other salts. All nutrient solutions uniformly contained iron, boron, manganese, and zinc at 0.5 p.p.m. and copper at 0.125 p.p.m.

Indoleacetic acid (Merck and Company) and alpha naphthalene acetamide (American Chemical Paint Company) were employed as the growth substances. They were used individually in series of concentrations ranging from 10^{-4} to 10^{-11} by multiples of ten at the several levels of nutrition. Fresh stock solutions of the growth substances were prepared for each watering. The proper amount of these solutions was mixed with the nutrient to give the desired concentration immediately before watering the plants. Applications were given three times a week between the first treatment, 10 days after planting, and harvest at the flowering stage. The volume of nutrient solution was increased as the plants matured, so that there was always a slight run off through the pot drain after each application.

Fresh and dry weights were taken for the tops, and dry weights for the roots. The roots were freed of sand by washing over a screen, dipping in saturated salt water, and rinsing in clear water. The material was dried for 24 hours in a well-ventilated draft oven at 80°C . Samples removed from the oven were kept in desiccators until weighed.

The experiments were designed on a factorial system, with treatments randomized within blocks; and an analysis of variance (11) was used to establish the significant points regarding top dry weight, root dry weight, and total plant dry weight.

Investigation

INDOLEACETIC ACID

EXPERIMENT I.—This ran from October 10 to November 7, 1940. During this period 60 per cent of the days were sunny and growing conditions were good. Plants reached the flowering stage in 28 days after planting the seed. Nutrient levels of minus K, 1/18 K, 3/9 K, and 9/9 K were used. An analysis of variance of the dry weight of the tops indicated significant increases at indoleacetic-acid concentrations near 10^{-9} over plants receiving the same 9/9 K nutrient but without the growth substance. The differences in response between plants receiving and those not receiving the acid at the 3/9 K, 1/18 K, and minus K levels were not significant. There was a tendency for dry weight of roots to increase with increasing concentrations of the growth substance and for dry weight of the tops to decrease at the higher concentrations. Also a definite decrease in succulence was associated with decreasing amounts of potassium.

EXPERIMENT II.—A more extensive experiment was designed having six blocks, within each of which thirty-one treatments were randomized (table 1). There were eight plants to a treatment in each block, and thus forty-eight plants to a treatment in the entire experiment. The results of this experiment illustrate those of the first as well.

This experiment was carried on between January 24 and February 25, 1941. Only 40 per cent of the days were sunny, there being less light than in the autumn. Thirty-two days were required for flowering. During this time ten applications of nutrient and growth substance were given. The greenhouse temperature averaged 70° F., and the average relative humidity ranged from 60 to 70 per cent.

No epinastic response was noted, even at the 10^{-4} and 10^{-5} concentrations after applications. This was in contrast to phosphorus-deficient and boron-deficient bean plants grown at the same time and receiving the same indoleacetic-acid concentrations, for these showed definite epinastic curvatures of the primary leaves. The first trifoliate leaves of the plants at the 10^{-4} concentration were very pale green compared with the 10^{-5} or any other treatment. This partial chlorosis persisted in the second and third trifoliate leaves as they appeared. Likewise the first obvious signs of stunting appeared at this concentration. By the eighteenth day after planting (twelfth after emergence), height differences were marked enough to distinguish clearly between the taller plants of the upper two potassium levels and the shorter plants of the lower two.

The first visible symptom of potassium deficiency appeared on the nineteenth day after planting in the minus K treatments. It required 6 additional days for this condition to become obvious in the 1/18 K plants. This first symptom was shown by the action of the pulvinus at the base of the primary leaf blade. Those

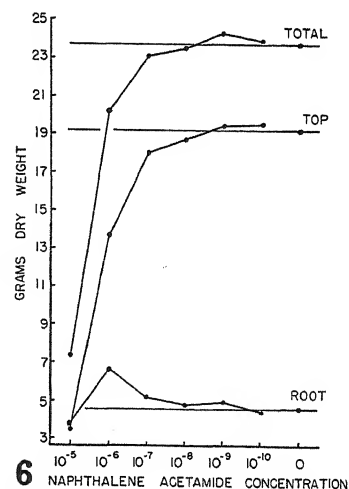
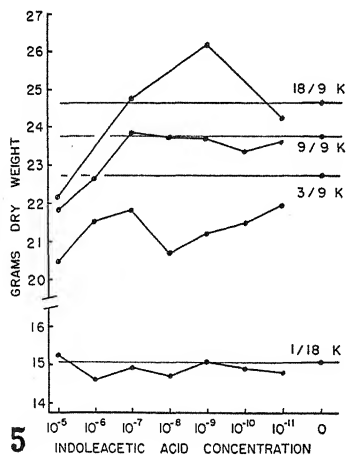
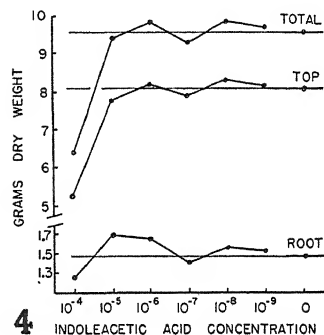
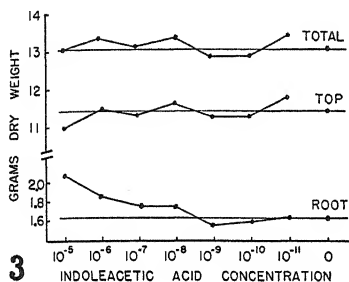
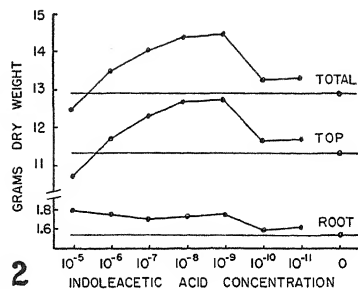
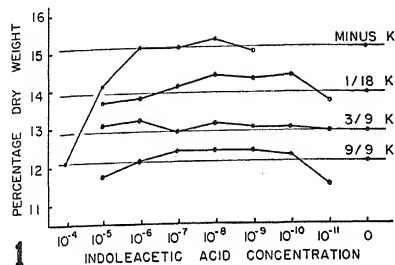
leaf blades which would a short time later exhibit the characteristic marginal chlorosis of deficiency would retain the nocturnal pendant position during the day. All others remained horizontal except at night. The early marginal yellowing of the deficient leaf blades was followed by more extensive chlorosis, until the entire leaf blade was chlorotic. The veins were the last portion so affected. By the time of harvest the primary leaves had begun to absciss and the first and even second trifoliate leaves were somewhat mottled with chlorotic areas.

By the twenty-seventh day after planting, flower buds were evident on all plants regardless of treatment. At harvest on the thirty-second day a few of these buds had commenced to open. Leaf size varied with concentration of growth substance supplied. The more stunted plants at the higher concentrations had considerably less leaf area than had those plants receiving a more favorable concentration. All plants, regardless of treatment, had at the same nutrition level the same total number of leaves.

The percentage dry weights for the bean tops are shown in figure 1. Although the range of the percentages is only slightly over 3.5 per cent, there does seem a clear-cut response to each nutritional level, and this response in a definite order. Succulence increases with increasing amounts of potassium. With the exception of the two highest concentrations of indoleacetic acid at minus K, all the points are well stratified. There seems little relationship between quantity of growth substance and percentage dry weight over the central range of the concentrations used. However, the rapid drop in percentage dry weight with the greatest amount of indoleacetic acid suggests that high concentrations may have a more measurable effect. Increases in succulence as a result of supplied growth substance have been reported, and it appears likely that they were associated with relatively high concentrations.

The dry-weight data of total plant, top, and root represent average weights for each treatment (table 1). An analysis of variance to establish the differences between these means necessary for significance at probabilities of 5 per cent and 1 per cent gave the following results. Mean differences are significant if greater than 1.00 gm. at 5 per cent and 1.32 gm. at 1 per cent probability for dry weight of total plant; 0.47 gm. at 5 per cent and 0.62 gm. at 1 per cent probability for dry weight of tops; and 0.22 gm. at 5 per cent and 0.29 gm. at 1 per cent probability for dry weight of roots.

A comparison of the nutrition levels, aside from the effect of growth substance, may be made from the controls where no indoleacetic acid was used. The dry weights for the roots indicate no significant differences among the four levels. In regard to dry weight of total plant and tops, however, these four levels differ significantly at 1 per cent probability in all comparisons except that of the 3/9 K with the 9/9 K level. These two are nearly alike in actual weights. During growth



FIGS. 1-6.—Each straight horizontal line represents the value for the specific control treatment which received no growth substance, for comparison with the lots which did. Fig. 1, percentage dry weight of bean tops at four levels of potassium. Fig. 2, dry weights for total plant, top, and root, at 9/9 K level (experiment II). Fig. 3, dry weights for 3/9 K level (experiment II). Fig. 4, dry weights for minus K level (experiment II). Fig. 5, dry weights of total plant at four potassium levels of experiment III. Fig. 6, dry weights for 9/9 K level of experiment IV.

both appeared much the same. Neither showed potassium deficiency symptoms nor any differentiating peculiarities. If based on the control-treatment results, the minus K, 1/18 K, and combined upper two levels seem to constitute the only distinct conditions. Yet a marked difference appears between the 9/9 K and 3/9 K levels in their response to the indoleacetic-acid concentration series (table 1; figs. 2, 3). At 10^{-9} the 9/9 K level yields 12.5 per cent increase in total dry weight over the control at that level, while the same concentration of growth substance with the 3/9 K level is slightly below its corresponding control. The 9/9 K level has a definite curve of response, with 10^{-7} , 10^{-8} , and 10^{-9} concentrations being significant increases and the latter two at 1 per cent probability, while the trend of

TABLE 1

DRY WEIGHT OF TOTAL PLANT, TOP, AND ROOT. FIGURES REPRESENT MEAN WEIGHT IN GRAMS OF SIX SAMPLES OF EIGHT PLANTS EACH

NUTRITION LEVEL AND PLANT PART		INDOLEACETIC-ACID CONCENTRATION							CON- TROL (NO ACID)
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	
9/9 K	Total plant.....	12.50	13.50	14.03*	14.44†	14.49†	13.27	13.35	12.88
	Top.....	10.70†	11.74	12.32†	12.70†	12.73†	11.68	11.72	11.35
	Root.....	1.79*	1.76*	1.71	1.74	1.76*	1.59	1.62	1.53
3/9 K	Total plant.....	13.00	13.26	13.07	13.28	12.76	12.81	13.32	12.99
	Top.....	10.94	11.40	11.32	11.53	11.21	11.23	11.68	11.35
	Root.....	2.07†	1.86	1.75	1.75	1.55	1.59	1.64	1.64
1/18 K	Total plant.....	10.92	10.94	11.91	11.96	11.22	11.63	11.15	11.34
	Top.....	9.18*	9.29	10.25*	10.16	9.60	9.93	9.58	9.70
	Root.....	1.74	1.64	1.66	1.79	1.62	1.70	1.57	1.64
Minus K	Total plant.....	6.48†	9.42	9.80	9.28	9.86	9.69	9.57
	Top.....	5.24†	7.71	8.14	7.87	8.30	8.14	8.09
	Root.....	1.24*	1.70	1.66	1.41	1.56	1.55	1.48

* Significant difference at 5 per cent probability from control at same nutrition level.

† Significant difference at 1 per cent probability from control at same nutrition level.

the 3/9 K level is irregular with no significant points. In this case plants appearing alike but receiving different nutrition responded in a decidedly different manner to indoleacetic-acid applications.

Much the same reaction occurs in relation to dry weight of tops. At 9/9 K significant increases are found at concentrations of 10^{-7} , 10^{-8} , and 10^{-9} , while the 10^{-5} concentration is depressed relative to the control. In the 3/9 K level no point is significant, either above or below the control. Only when root weights are considered does a similarity of response appear. At 10^{-5} concentration, root weights are significantly increased at both levels. A trend for increased root weight with the higher concentrations of indoleacetic acid is indicated.

The 1/18 K and minus K levels exhibited decided potassium deficiency symptoms and differed only in their intensity. Few significant weight differences were obtained at these levels (table 1; fig. 4). The 1/18 K level has significant decrease

at 10^{-5} and increase at 10^{-7} for the tops. On the basis of total plant weight, however, these points are not significant. No definite trend in relation to the indoleacetic-acid concentrations appears at either level. This irregular response does not indicate that indoleacetic acid can replace potassium or lessen the effect of potassium deficiency, as judged by the response of the plant as a whole.

Significant decreases in all three weights at the 10^{-4} treatment provide quantitative evidence for the stunting effect of growth substance under conditions of high concentration. This depression of growth probably would have occurred at any of the nutrition levels with a similar high concentration.

EXPERIMENT III.—To obtain data under growing conditions of higher light intensity, an experiment designed like the one just described but with eight blocks instead of six, and twenty-nine treatments randomized within each block, was carried out between March 17 and April 17, 1941. In this one the minus K level and the 10^{-4} indoleacetic-acid concentration were omitted and an 18/9 K level included. Sixty per cent of the days were sunny, and at noon on clear days light intensity in the greenhouse reached 5500–6000 foot candles. This was indicated in the increased dry weights. For example, the total dry weight of the control plants at the 9/9 K level was 85 per cent greater in this experiment than in experiment II.

Procedure in the two experiments was identical. Ten applications of the various levels of nutrient and growth substance were given. The plants were harvested when the flower buds were well developed, 31 days after planting the seed. Again there were no epinastic curvatures following indoleacetic-acid applications. The pendant position during the day of the primary leaf blades of potassium-deficient plants was observed as the first symptom of such deficiency. Flower buds appeared at the same time on all treatments. The total number of leaves per plant was the same at each nutrition level, regardless of treatment. Noticeable differences were apparent in leaf size and length of stem and petiole. Percentage dry weight decreased with increasing potassium, and the 18/9 K level averaged about 1 per cent lower than the 9/9 K level.

One condition was observed which was not detected in experiment II. On the last days before harvest the primary leaves of many of the 3/9 K level plants showed definite symptoms of potassium deficiency. The environmental conditions resulted in increased growth which apparently brought out the deficiency at this level.

Dry weights of total plant, top, and root were analyzed as before (table 2). Mean differences are significant for dry weight of total plant if greater than 1.46 gm. at 5 per cent probability and 1.93 gm. at 1 per cent probability; for dry weight of top if greater than 0.91 gm. at 5 per cent probability and 1.20 gm. at 1 per cent probability; and for dry weight of root if greater than 0.62 gm. at 5 per cent probability and 0.82 gm. at 1 per cent probability.

Root weights indicate few important differences and no obvious trends relative

to growth substance treatment. The control at $1/18$ K is markedly lower than the three higher levels, which cannot be separated among themselves.

Dry weights of tops parallel dry weights of total plant (fig. 5). Consideration of these dry weights of entire plants in the control treatments to observe the effect of the nutrition levels without the growth substance indicates three conditions. The $1/18$ K level is significantly lower than the other three. By harvest it exhibited pronounced deficiency symptoms. The $3/9$ K level is lower than the $18/9$ K level but does not vary significantly from the $9/9$ K level. The $3/9$ K plants developed the early deficiency symptoms just before harvest. The $9/9$ K and the $18/9$ K levels cannot be separated on the basis of dry weight of controls.

TABLE 2

DRY WEIGHT OF TOTAL PLANT, TOP, AND ROOT. FIGURES REPRESENT MEAN WEIGHT IN GRAMS OF EIGHT SAMPLES OF EIGHT PLANTS EACH

NUTRITION LEVEL AND PLANT PART		INDOLEACETIC-ACID CONCENTRATION							CON-TROL (NO ACID)
		10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	
18/9 K	Total plant.....	22.14†	24.71	26.13*	24.23	24.62
	Top.....	18.17†	20.47	21.60*	20.04	20.50
	Root.....	3.98	4.24	4.54	4.20	4.12
9/9 K	Total plant.....	21.81†	22.64	23.83	23.70	23.68	23.34	23.58	23.70
	Top.....	17.84†	18.80*	19.97	20.05	19.88	19.73	19.70	19.84
	Root.....	3.97	3.85	3.87	3.66	3.80	3.62	3.87	3.95
3/9 K	Total plant.....	20.44†	21.50	21.80	20.68†	21.16*	21.45	21.95	22.73
	Top.....	16.34†	17.62*	17.93	17.39†	17.51*	17.87	18.05	18.63
	Root.....	4.09	3.88	3.87	3.29*	3.64	3.58	3.90	4.10
1/18 K	Total plant.....	15.26	14.63	14.92	14.70	15.08	14.94	14.80	15.06
	Top.....	12.03	12.05	12.32	11.99	12.41	12.22	12.23	12.28
	Root.....	3.23	2.58	2.60	2.72	2.67	2.71	2.57	2.78

* Significant difference at 5 per cent probability from control at same nutrition level.

† Significant difference at 1 per cent probability from control at same nutrition level.

When the response of the $9/9$ K and $18/9$ K levels to indoleacetic acid is compared by dry weight of total plant, differences appear. The $18/9$ K level yields a significant increase over its control at 10^{-9} concentration and a decrease at 10^{-5} . The $9/9$ K trend has only the significant decrease at 10^{-5} . Obviously more potassium was available in the $18/9$ K level than in the $9/9$ K level during growth of the plants. Although neither showed decided differences in growth of controls, this higher amount of potassium at the $18/9$ K level may have been in part responsible for the increase of growth resulting from applications of indoleacetic acid. The two nutrient levels which in experiment II showed little difference in growth of controls and yet different response to indoleacetic acid were the $9/9$ K and the $3/9$ K. Of these the $9/9$ K level yielded the significant increases. Because of much less growth in this test, a similar relationship between potassium supply and plant

growth may have existed in this 9/9 K level as in the 18/9 K level just described. In both cases the dry weight increases correlated with indoleacetic-acid treatment were associated with nutrition levels of relatively high potassium.

The facts that all indoleacetic-acid treatments at the 3/9 K level fall below their control, and that scattered points in this curve are significantly below, suggest that this growth substance may hasten the appearance of potassium deficiency. Symptoms of this deficiency were just beginning to be apparent at harvest, but observations were not recorded on their relative intensity in treated and control plants. No decided differences were obtained between the indoleacetic-acid treatments and control at the 1/18 K level.

TABLE 3

DRY WEIGHT OF TOTAL PLANT, TOP, AND ROOT. FIGURES REPRESENT MEAN WEIGHT IN GRAMS OF SIX SAMPLES OF EIGHT PLANTS EACH

NUTRITION LEVEL AND PLANT PART		NAPHTHALENE-ACETAMIDE CONCENTRATION							CON- TROL (NO ACETAM- IDE)
		10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	
9/9 K	Total plant.....	7.41†	20.22†	23.06	23.44	24.24	23.83	23.62
	Top.....	3.52†	13.63†	17.92†	18.70	19.36	19.45	19.13
	Root.....	3.89*	6.61†	5.14*	4.74	4.88	4.38	4.40
3/9 K	Total plant.....	7.27†	16.40†	21.93	22.60	21.87	21.83	21.79	21.87
	Top.....	3.47†	10.56†	17.00	17.90	17.60	17.55	17.57	17.52
	Root.....	3.80	5.84†	4.93	4.72	4.26	4.28	4.22	4.35
1/18 K	Total plant.....	6.34†	13.54†	14.75	16.27	15.93	15.30	16.19	15.46
	Top.....	3.00†	8.82†	11.50*	12.78	12.52	12.27	12.73	12.34
	Root.....	3.34	4.72†	3.25	3.49	3.40	3.03	3.47	3.12
Minus K	Total plant.....	5.80†	11.09*	11.63	12.10	12.19	11.75	12.30	12.30
	Top.....	3.05†	6.88†	8.84	9.62	9.60	9.39	9.95	9.64
	Root.....	2.76	4.22†	2.79	2.48	2.59	2.37	2.35	2.66

* Significant difference at 5 per cent probability from control at same nutrition level.

† Significant difference at 1 per cent probability from control at same nutrition level.

ALPHA NAPHTHALENE ACETAMIDE

EXPERIMENT IV.—This experiment was carried out in the greenhouse under similar conditions and at the same time as experiment III. Experiment IV had thirty-one treatments randomized in six blocks, replicated six times. Potassium levels of 9/9 K, 3/9 K, 1/18 K, and minus K were employed, as were acetamide concentrations of 10^{-5} to 10^{-11} (table 3). All plants at the 10^{-5} concentration were much stunted and after eight applications of the nutrients and growth substance had practically ceased growth. The experiment was therefore harvested on the twenty-ninth day after planting.

A pronounced downward curling of the primary leaves at all 10^{-5} concentrations occurred shortly after the first application of naphthalene acetamide. This per-

sisted permanently throughout the experiment, and these plants, regardless of potassium level, remained stunted. Their second internodes elongated little and only one small trifoliate leaf, if any, expanded. Plants at all other concentrations at a given potassium level had the same number of leaves as the control at that level, although there were noticeable reductions in leaf size and stem and petiole elongation at the higher concentrations.

Potassium deficiency in this experiment, as in the others, was first indicated by the pendant position of the primary leaf blades during the day. By the time of harvest chlorosis due to potassium deficiency was evident in the second trifoliate leaves in the minus K plants. Although the flower buds were still extremely small at harvest, there appeared no hastening of their development at this stage by any of the naphthalene-acetamide treatments.

Average dry weights of total plant, top, and root were analyzed (table 3). Differences between means of dry weights greater than 1.14 gm. for total plant, 0.83 gm. for tops, and 0.58 gm. for roots are significant at 5 per cent probability. At 1 per cent probability these mean differences must be greater than 1.50 gm. for total plant, 1.10 gm. for tops, and 0.76 gm. for roots.

The control treatments provide a basis for judging the effect of the four nutrient levels without any naphthalene acetamide. The total plant average weights and averages for tops both differ sufficiently so that each level is clearly separated from the others. The dry weights of roots fall into two groups. The 9/9 K and 3/9 K levels cannot be separated between themselves but they do differ significantly from the 1/18 K and minus K levels, which in turn cannot be separated. Yet the weights for roots decrease consistently with decreasing potassium, so the four levels appear to constitute four distinct nutritional conditions.

Examination of the significant differences between the naphthalene-acetamide treatments and the control at any given level shows that these differences are present only at the higher concentrations. For total plant dry weight they are only decreases, and the same is true for dry weight of tops. The only significant increases in the entire experiment are for dry weight of roots, and these increases are well localized at the 10^{-6} concentrations. At harvest the roots at these treatments were more fibrous and extensive than at others.

The response at any one nutrition level is the same in general trend as that at any other of the four levels tested (fig. 6). The difference appears only in degree, in that the weights are progressively less as potassium supply decreases.

The percentage increase in dry weight of root at the 10^{-6} concentration of naphthalene acetamide over the control at the same level is 47.2 at 9/9 K, 34.3 at 3/9 K, 51.3 at 1/18 K, and 58.6 at minus K. These increases occur in treatments associated with reduction in dry weight of tops. This decidedly alters the ratio of

tops to roots. At the 9/9 K level the dry weight of tops of the control treatment is approximately 4.3 times that of the roots. At the same nutrition level, but with naphthalene acetamide supplied in the nutrient at 10^{-6} concentration, the dry weight of the tops is only 2.1 times that of the roots.

Discussion

When different responses are obtained in nutritional work in which the solutions have been altered by the substitution of a salt, the question arises as to whether the effects are attributable to the altered essential element or to the substituted ion. Such inquiry may well be made in the experiments with indoleacetic acid reported here. The only nutrient level in experiment II which gave significant increases with indoleacetic-acid treatment was that of 9/9 K, which received no sodium after treatments commenced. Perhaps sodium prevented a similar response at the other nutrient levels where it was used as a substitute ion for potassium. But in experiment III, with much greater plant growth, the 9/9 K level failed to give significant increases. Presence of sodium could not have resulted in this response. In this experiment the 18/9 K level, with chlorine as a foreign ion and with higher potassium, did yield a significant increase in dry weight. Yet the increases of experiment II at 9/9 K were obtained on plants watered with solutions containing no chlorine. Sodium and chlorine may influence the response, but it seems unnecessary to consider the absence of sodium or the presence of chlorine of particular importance in obtaining increased growth with indoleacetic-acid applications.

The evidence seems to favor the view that variation in potassium supply rather than variation in the substituted or added ion was chiefly responsible for the different reactions at the several levels. If this is granted, it appears that relatively high potassium is necessary to obtain stimulating effects on growth of the total plant with indoleacetic acid. High potassium plants may be limited in growth by a relative deficit of growth substance. The addition of indoleacetic acid may raise this limitation and increased growth result. At the lower potassium levels this chemical element may be the limiting factor; thus greater supply of indoleacetic acid results in no growth increase.

GRACE (5) has studied the effect of several chemicals used with indoleacetic acid in a talc carrier on the rooting of stem cuttings. He concluded that potassium acid phosphate in combination with indoleacetic acid has decided effects on rooting.

In these experiments, whenever stimulation of total plant dry weight resulted from indoleacetic-acid treatment, it was observed in the same general region of concentrations, 10^{-7} , 10^{-8} , and 10^{-9} . Yet the much different response over the

entire range of concentrations suggests the value of employing several concentrations in experiments with growth substances.

Studies of the response of kidney bean plants to emulsion sprays of naphthalene acetamide have been made by KRAUS and MITCHELL (7) and by MITCHELL and STEWART (10). The latter observed the effects of four concentrations of emulsion spray as judged by dry-weight analysis 10 days after spraying. They reported root increases of 48 per cent with the most concentrated spray. Both papers mentioned the more fibrous nature of the roots and the suppression of top growth with concentrated sprays. These observations are similar to those reported in this paper for the 10^{-6} concentration, although in this work the naphthalene acetamide was applied in the nutrient solution and the plants were not harvested until flower buds were evident. No data were obtained concerning the relative efficiency of the increased root system of plants treated with naphthalene acetamide.

Summary

1. Red kidney bean plants grown in sand culture at different levels of potassium nutrition and receiving indoleacetic acid or alpha naphthalene acetamide in the nutrient solution in a series of concentrations were compared by dry weights of top, of root, and of total plant.

2. Plants receiving different nutrition but externally appearing alike responded in a markedly different manner to indoleacetic-acid treatment.

3. Relatively high potassium in the nutrient solution was necessary to obtain increased dry weight of total plant by indoleacetic-acid treatment.

4. Under certain levels of nutrition, increases as great as 12.5 per cent in total plant dry weight were obtained by indoleacetic acid supplied at the 10^{-9} concentration. Significant increases were obtained at concentrations of 10^{-7} , 10^{-8} , and 10^{-9} .

5. Indoleacetic acid appeared unable either to replace potassium or to enhance its utilization under deficiency conditions, as judged by total plant response.

6. The time of flowering was not hastened by indoleacetic-acid treatment, nor was there any indication that naphthalene acetamide accelerated the appearance of flower buds.

7. The number of leaves per plant was not increased by either growth substance, although leaf size varied considerably.

8. Percentage dry weight increased as potassium decreased, and only at relatively high concentrations of indoleacetic acid (10^{-4}) was increased succulence noted.

9. Plants at four potassium levels yielded the same general trend of response to naphthalene-acetamide treatment. They differed only in the degree of this response, in that dry weights were less with decreasing potassium.

10. Dry weights of roots increased 34-58 per cent at the 10^{-6} concentration of naphthalene acetamide. At this same concentration growth of tops was significantly reduced.

11. The first visible potassium deficiency symptom was expressed by the primary leaf blades retaining the nocturnal pendant position during the day.

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LITERATURE CITED

1. AVERY, G. S., JR., BURKHOLDER, P. R., and CREIGHTON, H. B., Plant hormones and mineral nutrition. *Proc. Nat. Acad. Sci.* 22:673-678. 1936.
2. ———, Nutrient deficiencies and growth hormone concentration in *Helianthus* and *Nicotiana*. *Amer. Jour. Bot.* 24:553-557. 1937.
3. EATON, F. M., Interrelations in the effects of boron and indoleacetic acid on plant growth. *BOT. GAZ.* 101:700-705. 1940.
4. GRACE, N. H., Physiologic curve of response to phytohormones by seeds, growing plants, cuttings, and lower plant forms. *Canad. Jour. Res. Sec. C.* 15:538-546. 1937.
5. ———, Effects of potassium acid phosphate, cane sugar, ethyl mercuric bromide, and indolylacetic acid in a talc carrier on the rooting of stem cuttings. *Canad. Jour. Res. Sec. C.* 19:99-105. 1941.
6. HWANG, Y., and PEARSE, H. L., The response of seeds and seedlings to treatment with indolylacetic acid. *Ann. Bot. n.s.* 4:31-38. 1940.
7. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. *BOT. GAZ.* 101:204-225. 1939.
8. McROSTIE, G. P., HOPKINS, J. W., and GRACE, N. H., Effect of phytohormone dusts on growth and yield of winter wheat varieties. *Canad. Jour. Res. Sec. C.* 16:510-515. 1938.
9. MITCHELL, J. W., and HAMNER, C. L., Stimulating effect of beta(3)indoleacetic acid on synthesis of solid matter by bean plants. *BOT. GAZ.* 99:569-583. 1938.
10. MITCHELL, J. W., and STEWART, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. *BOT. GAZ.* 101:410-427. 1939.
11. PATERSON, D. D., Statistical technique in agricultural research. McGraw-Hill, New York. 1939.
12. PEARSE, H. L., Effect of phenylacetic acid on the growth of tomato plants. *Nature* 138:363-364. 1936.
13. ———, The effect of phenylacetic acid and of indolebutyric acid on the growth of tomato plants. *Jour. Pomol. and Hort. Sci.* 14:365-375. 1936.
14. SMITH, ORA, NASH, L. B., and DAVIS, G. E., Chemical and histological responses of bean plants grown at different levels of nutrition to indoleacetic acid. *BOT. GAZ.* 102:206-216. 1940.
15. SKOOG, FOLKE, Relationships between zinc and auxin in the growth of higher plants. *Amer. Jour. Bot.* 27:939-951. 1940.
16. TEMPLEMAN, W. G., The effect of some plant growth-substances on dry-matter production in plants. *Empire Jour. Exp. Agr.* 7:76-88. 1939.
17. THIMANN, K. V., and LANE, R. H., After-effects of the treatment of seed with auxin. *Amer. Jour. Bot.* 25:535-543. 1938.

ANATOMY AND SORUS DEVELOPMENT OF CYSTOPTERIS BULBIFERA

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(WITH TWENTY-FIVE FIGURES)

Introduction

The genus *Cystopteris* has given difficulty in classification. DIELS (5) placed it in the Woodsieae, but SCHLUMBERGER (8) excluded it from this tribe. BOWER (1) placed the genus among the Genera Incertae Sedis, but states that *Cystopteris* ranks nearer the Cyatheoid than the Davallioid derivatives, and that its origin is superficial as in the Woodsioid and Dryopteroid ferns. In a later publication (2) he states that the question of ancestry is between the Pteroid type with marginal, or the Cyatheoid type with superficial sori. More recently CHRISTENSEN (4) has placed *Cystopteris* in the Asplenoideae, between the Blechnoideae and Dryopteroidae.

Previously only two species, *Cystopteris fragilis* (L.) Bernh. and *C. montana* (Lam.) Bernh., have been examined morphologically and anatomically. The present investigation of *C. bulbifera* (L.) Bernh. was undertaken in the hope that further knowledge of the anatomy and morphology of this species would aid in establishing the relationships of the genus.

Material and methods

Leaves and rhizomes were collected at the base of the eastern side of Mount Toby, Massachusetts. Material collected in May was used for studying the origin and development of the sorus. The young fronds were only partly unfolded; the tips which bore very young sori were still encased by the rest of the frond. Material collected in October was used for studying the rhizome and root. Portions of root, rhizome, petiole, pinnae, and young leaves were killed and fixed in a chromo-acetic solution of 1 per cent chromic acid and 2 per cent acetic acid and were then imbedded in paraffin. Most sections were stained in safranin-light green or in safranin-gentian violet combinations. Sections of early stages of the sorus were stained in Heidenhain's iron-alum haematoxylin and counterstained in safranin.

Observations

RHIZOME.—The rhizome develops radially, with leaves diverging from all sides. Because of the closeness of the leaf bases to one another, the rhizome gives the appearance of being merely a spiral succession of imbricated bases. Although the

greater part of the rhizome is made up of leaf bases, the central portion contains a dictyostele with curving meristeles bordering the long leaf gaps. Transverse sections of the rhizome show the number of meristeles ordinarily to be either three or four, although there are occasionally five. The binary leaf traces found in the leaf base arise individually, each from a separate meristele. Each leaf is accompanied by a single root. The trace which enters the root diverges near the base of the leaf gap. This base is formed by the divergence of a single meristele into two strands, each of which may give off a leaf trace before anastomosing with an adjacent meristele (fig. 1, rt_1 , lt_1), or—more often—one will give off a leaf trace before anastomosing but the other will merge with an adjacent strand before giving off a leaf trace (fig. 1, rt_4 , lt_4). The leaf traces, usually diverging in the lower half of the leaf gap, do not always arise at the same distance from the base of the gap, nor at the same level.

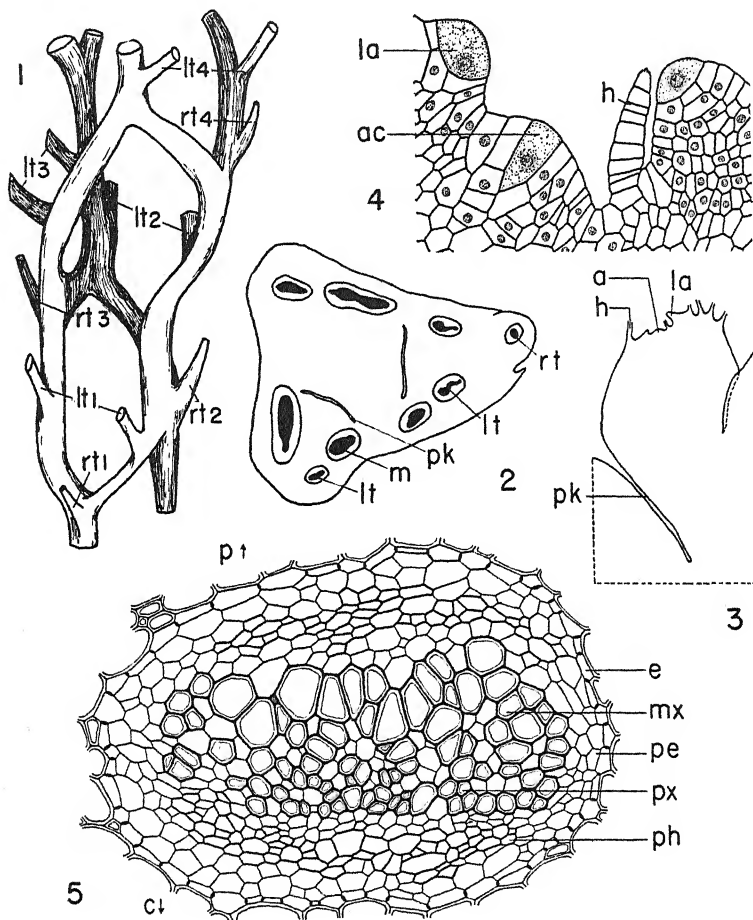
Pockets in the rhizome are formed between the adaxial side of the leaf base and the rhizome itself, and extend down into the pith region, often extending below the point at which the leaf traces diverge from the meristeles into the leaf base (figs. 2, 3). GWYNNE-VAUGHAN (7) describes similar pockets in *C. fragilis* as large, downward ranging into internal ground tissue, and limited to three in any cross section.

The apex of the rhizome is protected by scales, multicellular hairs, and glandular hairs, as well as by the petioles of young leaves. The large apical cell is wedge-shaped, with three cutting faces (fig. 4). Each meristele is surrounded by a continuous endodermis, the cells of which show a Casparian strip on the radial walls. A pericycle, two or three cells thick, lies between the endodermis and the phloem. The central core of xylem, oval in transverse section, is surrounded by parenchyma and, outside of that, by a band of phloem which is not always continuous. The isolated protoxylem cells are found near the cortical side of the xylem core, the larger metaxylem cells being toward the pith.

Each vascular strand of the leaf trace resembles a meristele but differs in the shape of the xylem strand. In transverse section the two traces are mirror images, with the protoxylem regions at the two ends of the traces partly inclosed by hooks of metaxylem (figs. 6, 7). In the rachis the two leaf traces merge, making a V-shaped trace, although the xylem strands retain in part their characteristic shape (fig. 8).

* **Root.**—The root trace diverges endogenously from a meristele, usually below but sometimes above the leaf gap. The primordium of a root may be seen near the rhizome tip, close to the base of the primordium of the leaf. The first protoxylem cells of the diarch stele appear after the root has progressed through the cortex far enough to break the epidermis. The course that the root may take when it is passing through the cortex of the rhizome varies. The root may grow obliquely

toward the tip but before becoming free turn back; or it may pass outward almost at right angles. When it breaks through the epidermis it may grow parallel to and in contact with the rhizome for some distance. The root cap is seven or eight cells thick just beyond the apex.

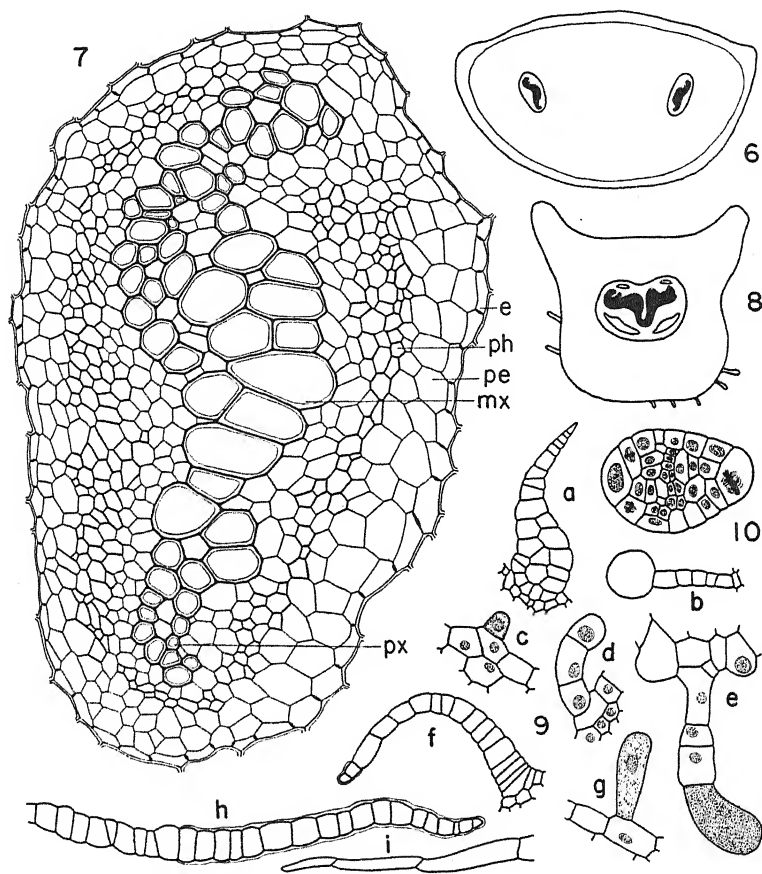


FIGS. 1-5.—Fig. 1, reconstructed vascular system: *lt*, leaf trace; *rt*, root trace. Fig. 2, diagram of cross section of mature rhizome. Fig. 3, same of longitudinal section of rhizome through apex: *a*, apex; *la*, leaf apex; *h*, hair or scale; *pk*, pocket; *m*, meristele. Fig. 4, apex of rhizome: *ac*, apical cell. Fig. 5, detail of meristele: *mx*, metaxylem; *px*, protoxylem; *pe*, pericycle; *e*, endodermis; *p*, region of pith; *pk*, phloem; *c*, region of cortex.

DERMAL APPENDAGES.—Types of appendages found on the sporophyte are scales, multicellular hairs, glandular hairs, and unicellular hairs (fig. 9). They occur on young parts as on the rhizome tip, petioles, rachis, and pinnae of young leaves, and on both the inner and outer surface of bulbils. They generally dis-

appear as these parts mature, but unicellular hairs—which may be slightly swollen at the tip—persist on the lower surfaces of the midribs of pinnae and pinnules.

LEAF.—The leaves are long, delicate, and light green in color. The mesophyll does not have a differentiated palisade layer, but there is more intercellular space

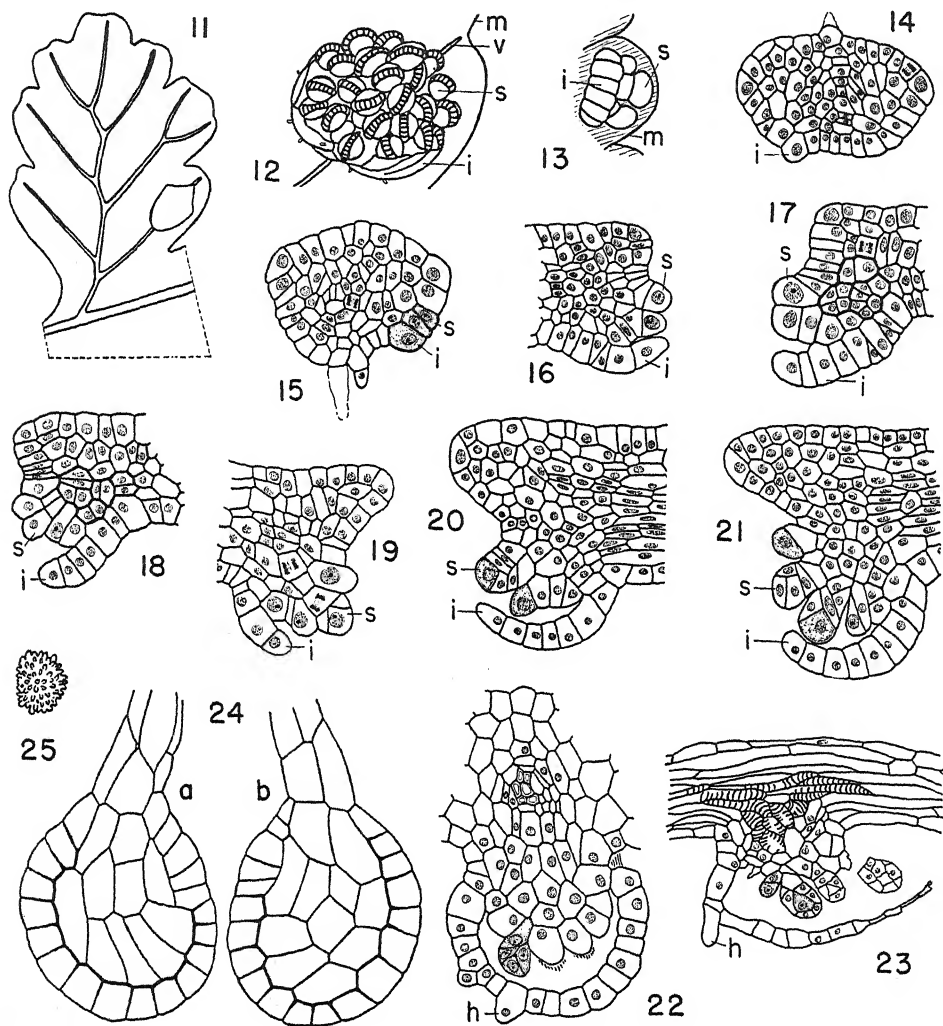


FIGS. 6-10.—Fig. 6, diagram of cross section of stipe. Fig. 7, detail of vascular bundle of stipe: *e*, endodermis; *ph*, phloem; *pe*, pericycle; *mx*, metaxylem; *px*, protoxylem. Fig. 8, diagram of cross section of rachis. Fig. 9, dermal appendages (*a-i*). Fig. 10, cross section of very young pinnule.

in the lower part than in the upper. Leaf primordia are very plump, as are also the pinnae as they arise from the rachis. The margin of each pinnule is composed of a row of cells with two cutting faces, which by continued divisions bring about the broadening of the pinnule (fig. 10). The pinnules bear two to eight sori near the outer margin of the small lobes. The sori appear while the pinnule is subterete.

SORUS.—In a mature leaf the sorus is seated near the margin of a pinnule on a

vein which extends beyond it. When young, the sorus is covered by a pocket-like indusium which opens on the side toward the margin of the pinnule (fig. 11). As soon as the first sporangia mature, the indusium begins to shrivel and eventually



FIGS. 11-25.—Fig. 11, pinnule with young sorus. Fig. 12, mature sorus: *m*, margin of leaf; *v*, vein; *s*, sporangium; *i*, indusium. Fig. 13, very young sorus, surface view. Figs. 14-21, stages in the development of sorus. Fig. 22, section cut across vein through young sorus: *h*, hair. Fig. 23, section cut parallel with vein through young sorus. Fig. 24a, b, two faces of young sporangium. Fig. 25, mature spore.

exposes all the sporangia (fig. 12). As it shrivels it is apt to tear lengthwise, giving the appearance at maturity of a basal indusium made up of scales. The indusium bears unicellular hairs and is one cell in thickness, except at the base directly in the

center where it may be two cells thick. The bilateral symmetry of the sorus is exaggerated by lateral pouches of the indusium, which extend outward from the base and over the top of the receptacle (fig. 22). The axis of the receptacle is inclined toward the margin of the pinnule (fig. 23).

The first indication of the sorus is found while the pinna is composed of undifferentiated tissue and has broadened but slightly (figs. 14, 15). As seen in section, an initial of the indusium five or six cells back from the margin becomes enlarged and papillate. This is followed by two or three sporangial initials between the indusium and the marginal cell. Two or three anticlinal divisions may occur in the superficial cells between the sporangial initials and the margin, but these cells do not enlarge much at this time (figs. 16-18). The tissue of the receptacle is formed by repeated division of the cells just beneath the sporangial and indusial initials (figs. 17-19). The cells of the young indusium undergo periclinal divisions, forming a layer curving from the proximal side of the sorus toward the margin (figs. 13, 16-21). In early stages of the sorus, marginal growth of the leaf is practically at a standstill and the marginal cell is often forced by intercalary divisions in the soral region to a position almost on the plane of the upper surface of the leaf (figs. 16-18). Growth of the receptacle is greater on the proximal side of the sorus, and the new sporangial initials are formed between the oldest sporangium and the base of the indusium (figs. 16, 18, 20). One, rarely a second, sporangium is formed on the distal side of the receptacle (fig. 21). This shows a gradate sequence in sporangial development, modified by the suppression of receptacle growth on the distal side of the sorus. The sorus later becomes mixed. The marginal cell at all times remains completely distinct from the tissues of the sorus (figs. 16-18), although in early stages the growth of the receptacle on the proximal side, the more advanced development of sporangia on the distal side, and the suppression of activity in the marginal cell—all place the young sorus on what has been interpreted to be the margin of the leaf. By the time there are several sporangia in early stages of development, the more active marginal growth of the leaf places the sorus on the under side of the leaf (figs. 19-21). About this time maturation of the main vein of the leaflet begins, followed by differentiation of the lateral veins. In the early stages, provascular strands can be seen extending into the receptacle of the young sorus and into the leaf blade beyond (fig. 20).

Either the first or second division of the sporangial initial is usually oblique. The mature sporangium has a slightly oblique annulus of fourteen indurated cells interrupted by the three-rowed stalk. The cell between the base of the annulus and the stalk is longer than the annulus cells, and the walls are unthickened. The mature stalk is relatively stout, with irregularly inclined walls (fig. 24). The sporangium contains twenty-four to forty-eight spores, but the usual number is thirty-two. They are dark brown, without a perispore, and are covered with spine-like protruberances (fig. 25).

Discussion

In considering the relationships of *Cystopteris* the following points should be considered: its various vegetative characters—delicate habit, crowded leaves, dermal appendages, dictyostelic stem structure, and binary leaf traces with which one root is associated; its reproductive characters—the superficial position and origin of the sorus, its zygomorphic form, the pocket-like indusium, the gradate sequence changing to mixed, the three-rowed stalk of the sporangium, and the bilateral spore.

There is little that suggests relationship between *Cystopteris* and any group of the Marginales. The only point which would ally them is a marginal origin of the sorus. In *Cystopteris* the sorus is seated on a vein which sends a branch into the receptacle but continues beyond toward the leaf margin. In forms secondarily superficial (for example, *Davallia*) the origin of the sorus is definitely marginal on a flattened margin receptacle; the superficial position is assumed when the sporangia are somewhat well developed. In almost all cases the marginal cell of the leaf is active in the formation of the receptacle, sporangia forming on both sides of it; and the continuation of the lamina as upper indusium is a proliferation of the leaf tissue on the upper side of the marginal cell (1). In *Cystopteris*, however, the marginal cell continues to function as the marginal cell of the leaf and is not concerned in the formation of the sorus.

BOWER (3) finds that "the segmentation of the sporangia [in *C. fragilis*] is also a matter for note. It will be seen that the stalk is a relatively massive one with irregularly inclined walls. This . . . is in sharp contrast to the simple transverse segmentation of the stalk seen in *Davallia*."

The interpretation of the origin of the sorus as marginal may be attributed to the precocity of the pinnule in its soral development, since in ferns where the sori are obviously superficial (for example, *Asplenium*) the pinnule may become broadened and have a well-defined vein before the sporangia appear (1). In *Cystopteris* the development of the first sporangium precedes the broadening of the leaf and the development of the vascular tissue. At this stage the room for the sorus on the under surface is very limited. As the leaf broadens the almost marginal position is maintained for a short period by the great activity in the sorus in comparison with the slight activity of the margin, but the marginal cell at all times remains distinct from the sorus. The superficial position is definite before the vascular tissues are mature. The origin and development of the sorus have been observed by GOEBEL (6) in *C. montana* and by BOWER (3) in *C. fragilis*. Their figures indicate that a similar interpretation is reasonable for these two species.

In the Superficiales similarities can be found to members of the Onocleoidae, the Dryopteroidae, the Asplenioideae, and the Woodsiaeae. The smaller forms of the Dryopteroid group have a simplified dictyostele, and certain species (for ex-

ample, *D. phlegopteris* and *D. thelypteris*) have a binary leaf trace (1). The receptacle of the sorus in this group is horseshoe-shaped, and while there may be a very early tendency to gradate sequence of the sporangia, it is quickly replaced by a mixed condition. The sporangium has not lost all traces of the obliquity of the annulus, but it is not so noticeable as in *Cystopteris*. The stalk is three-rowed. *Dryopteris* differs from *Cystopteris* in the accentuated tilting and zygomorphy of the receptacle and in the early mixed sporangial sequence.

CHRISTENSEN's most recent classification (4) places *Cystopteris* in the Asplenoideae, and suggests a relationship to *Athyrium*. The adult stem of *Athyrium* contains a rather wide-meshed dictyostele. The petiole is traversed at its base by broad straps of the leaf trace, which unite below the blade to form a gutter-shaped meristele (1). The leaves are borne spirally. *Cystopteris* shows close resemblance in anatomy but differs in soral structure and in details of the sporangium, which has an annulus with an oblique tendency and relatively massive stalk in contrast to the incomplete vertical annulus and one-rowed stalk of the sporangium of *Athyrium*.

In the appearance, size, and texture of the fronds and in anatomy, *Cystopteris* shows a close resemblance to the Woodsiae. The vascular system of *Woodsia* is a simple dictyostele, with two leaf trace strands, with which one or more roots are associated (8).

Cystopteris forms a link in the series tracing the zygomorphy of the sorus from the complete radial sorus with basal indusium—found in some species of *Woodsia*—to the horseshoe-shaped sorus with the tilted receptacle and covered by an incomplete indusium found in *Dryopteris*. In the Woodsiae and *Dryopteris* the slightly oblique annulus is interrupted by the three-rowed stalk, and the spore output is 48–64. In the Woodsiae the sequence of sporangial development is initially basipetal, later becoming mixed as in *Cystopteris*; in *Dryopteris* the gradate sequence is merely suggested. *Cystopteris* seems to be intermediate between *Woodsia* and *Dryopteris*, but more closely allied to *Woodsia* than to *Dryopteris*.

Summary

1. The rhizome of *Cystopteris bulbifera* is radial, with leaves diverging from all sides. It contains an unperforated dictyostele with curving meristeles bordering the leaf gaps. In transverse section ordinarily three or four meristeles are found.
2. The strands of the binary leaf trace diverge individually in the lower half of the leaf gap, one from each side of the leaf gap, and merge in the rachis.
3. Each root arises singly and accompanies the leaf base, the root trace diverging near the base of the leaf gap.
4. Unicellular, multicellular, and glandular hairs, and also scales are present on

young parts of the sporophyte. Unicellular hairs persist on the lower surface of the rachis and of the midribs of the pinnae and pinnules.

5. The sorus originates on the pinnule while it is subterete. The sorus is superficial in origin; the indusium and sporangial initials appear a few cells back from the marginal cell, which continues its marginal activity and takes no part in the formation of the sorus. The sorus is seated anterior to the vein ending. Its receptacle has bilateral symmetry and is tilted toward the margin of the pinnule.

6. The sporangium has a slightly oblique annulus, which usually consists of fourteen cells interrupted by a three-rowed stalk. The sequence in development in the sorus is at first gradate but later mixed.

7. The closest affinities of *Cystopteris* seem to be with the Woodsieae.

The writers express their thanks to Professor ALMA G. STOKEY for helpful direction and criticism during the course of this study.

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LITERATURE CITED

1. BOWER, F. O., The ferns. Vol. III. Cambridge. 1928.
2. ———, Primitive land plants. London. 1935.
3. ———, Studies in the phylogeny of the Filicales. II. *Lophosoria*, and its relation to the Cyatheoideae and other ferns. Ann. Bot. 26:269-323. 1912.
4. CHRISTENSEN, CARL, Manual of pteridology. Edited by Fr. Verdoorn. The Hague. 1938.
5. DIELS, L., Polypodiaceae. In ENGLER and PRANTL's Die Natürlichen Pflanzenfamilien. 1902.
6. GOEBEL, K., Organographie der Pflanzen. Jena. 1930.
7. GWYNNE-VAUGHAN, D. T., On the possible existence of a fern stem having the form of a lattice-work tube. New Phytol. 4:211-216. 1905.
8. SCHLUMBERGER, OTTO, Familienmerkmale der Cyatheaceen und Polypodiaceen und die Beziehungen der Gattung *Woodsia* und verwandter Arten zu beiden Familien. Flora 102: 383-414. 1911.

GRASS STUDIES. V. OBSERVATIONS ON PROLIFERATION¹

ETLAR L. NIELSEN

(WITH SIX FIGURES)

Introduction

ARBER (1) defines proliferation in grasses as "the conversion of the spikelet, above the first glumes, into a leafy shoot." Reports covering a number of species wherein proliferations have been observed have been published (1, 2, 3, 5). The majority of these observations appear to have been made upon plants growing in areas of high altitudes, high latitudes, or in moist situations. The present study was made mainly upon plants growing under the relatively xeric conditions in northwest Arkansas during the summer months of the last few seasons, under very wet conditions of west-central Wisconsin, under greenhouse conditions, or under grazing.

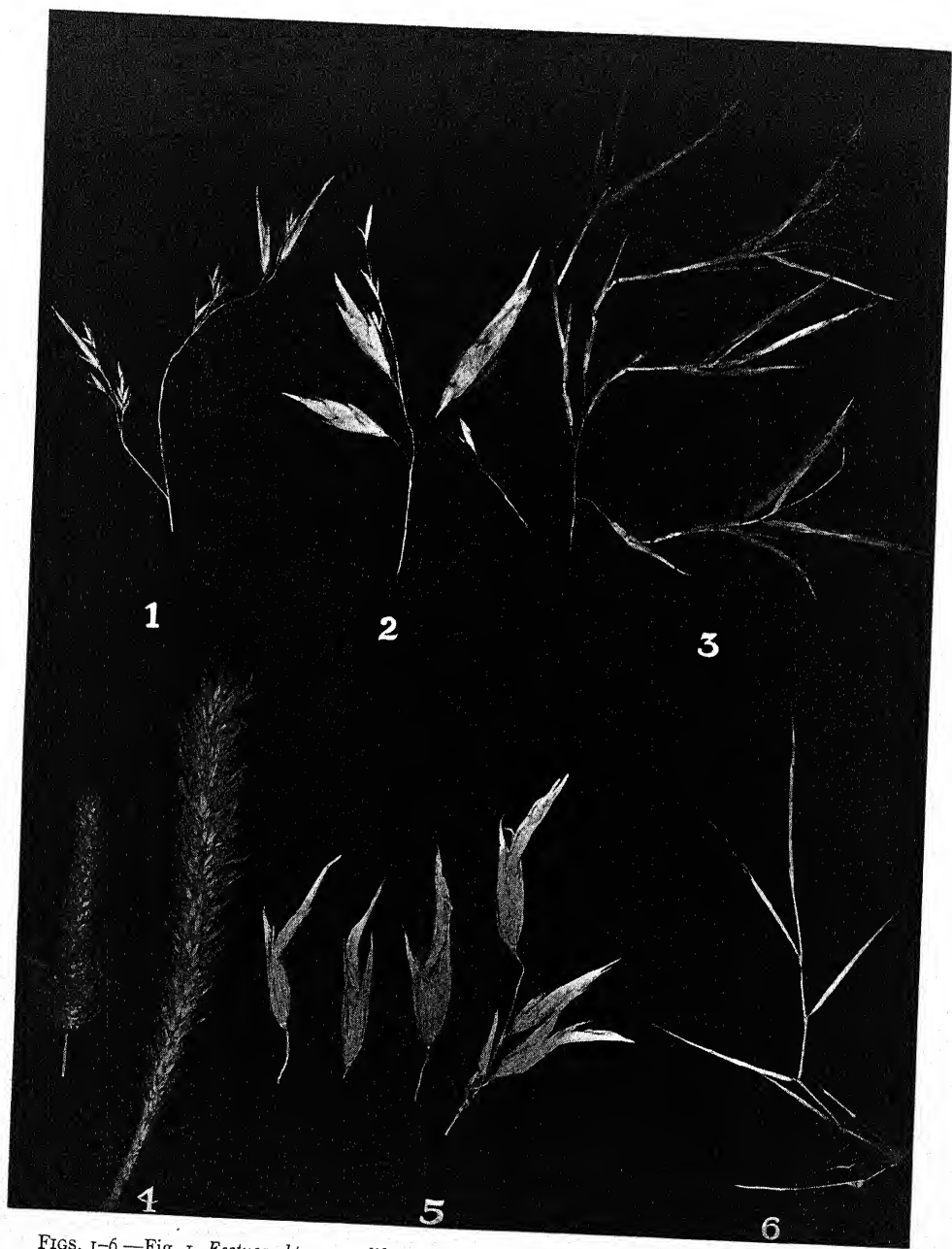
Observations

FESTUCA OBTUSA SPRENG.—A plant upon which a number of florets were proliferating was collected near Crosses, Madison County, Arkansas, in July, 1936. Subsequently other material of this same species bearing abnormal spikelets was collected from central and northwest Arkansas. With one exception the plants grew in dry and open situations. Examination of the cited herbarium material indicates that some spikelets of the panicles appear normal in every respect while others bear proliferating florets. In some spikelets only one floret developed. This proliferated and was subtended by a pair of glumes. In other instances, where two or more florets had developed, the lowermost appeared normal while the more distal member of the same spikelet had proliferated. No spikelets have been observed with more than one floret proliferating, however, nor were any normal florets observed to be borne more distal than the proliferating one (fig. 1).

Herbarium material.—Crosses, Madison Co.: *Nielsen*, no. 4045. Mt. Magazine, Logan Co.: *Nielsen* and *Younge*, no. 5952. White Rock Mountain, Franklin Co.: *Nielsen*, no. 6016. Devils Den State Park, near Winslow, Washington Co.: *Younge* and *Nielsen*, no. 5959 (Mr. J. R. Swallen comments concerning this specimen: "The one or two apparently normal spikelets seem to be *Festuca versuta* Beal. . . . However the plant is abnormal and since *F. obtusa* Spreng. is common in this region I believe it is more likely to be the latter species.").

BROMUS INERMIS LEYSS.—In this species essentially the same conditions ob-

¹ Research paper no. 692, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.



FIGS. 1-6.—Fig. 1, *Festuca obtusa*, proliferating and normal spikelets. Fig. 2, *Bromus purgans*, much enlarged spikelets but proliferations not well developed morphologically. Fig. 3, *B. inermis*, proliferations wholly replacing normal spikelets. Fig. 4, *Phleum pratense*, well-developed proliferations of spikelets replacing normal structures. Fig. 5, *Avena sativa*, spikelets with proliferating upper florets. Fig. 6, *Panicum virgatum*, proliferation that replaced entire panicle.

tained as were found in *Festuca obtusa*. In 1938, proliferation was observed in nine of twelve selections of smooth brome grass growing in the grass nursery at the Agricultural Experiment Station, Fayetteville, Arkansas. Nearly all the spikelets of the panicle of some plants were abnormal in development, whereas in other panicles of plants of the same selection only a few florets appeared to be affected. In most instances only a single floret of a spikelet proliferates. Although these selections have been under observation since 1937, this condition was observed in this species in 1938 only. Shortly after July 10, 1938, when this condition was observed at Fayetteville, the writer examined a number of areas of this species in Minnesota and Wisconsin. No proliferations were found at this time, nor were any observed in the aftermath growth on these same areas when subsequently examined on September 17 of the same year (fig. 3).

Herbarium material.—Grass nursery, University of Arkansas Experimental Farm, Fayetteville: *Nielsen*, no. 5595.

BROMUS PURGANS L.—During the fall of 1939 much-enlarged florets occurred abundantly on plants of this species situated on dry ledges and in exposed positions in a number of localities of northwest Arkansas. In none of the numerous plants examined were the proliferations as well developed as in the selections of *B. inermis* previously discussed, and—in contrast to the condition in the latter species—usually all the florets of a spikelet were abnormal; in most instances comparatively few normal florets were found on the affected plants (fig. 2).

Herbarium material.—Lake Wedington, Washington Co.: *Younge and Nielsen*, nos. 6007, 6011. Devils Den State Park, Washington Co.: *Younge and Nielsen*, nos. DD200, DD204.

PHLEUM PRATENSE L.—Only in 1938 were proliferating spikelets observed in the nearly mature inflorescences in four of nineteen selections of this plant grown continuously in the grass nursery at the station during the period 1937–1940. The first abnormalities were observed on July 15, and others subsequently appeared until July 25. Prior to June 1, moisture had been plentiful. A period of light rainfall and high temperatures followed. Here the combination of abundant moisture followed by severe drought appeared associated with the occurrence of proliferation.

Also in 1938, aftermath timothy meadows in the vicinity of Chippewa Falls, Wisconsin, were examined regularly during September and October. No proliferations were found until October 21. Those observed at that time were found on plants growing near the margin of a meadow situated in a lowland subjected that year to frequent inundation, or along a service road entering the meadow. All the abnormal inflorescences were either emerging or had just emerged from the boot. During 1938 the rainfall at Chippewa Falls had been abundant during the entire summer and autumn, and moderate temperatures had prevailed. In this instance

the phenomenon may have been induced by the short days and low temperatures of late autumn (fig. 4).

Herbarium material.—Grass nursery, University of Arkansas Experimental Farm, Fayetteville: selections 361, 365, 393. Chippewa Falls, Chippewa Co., Wisconsin (October, 1938): *Nielsen* (five specimens).

AVENA SATIVA L.—A proliferating specimen of the commercial variety Ferguson 922 was forwarded to the writer by Mr. Paul H. Millar of the Arkansas State Plant Board. He reported the abnormality as "occurring abundantly in very moist or wet situations in the oat fields of the rice belt near Lonoke, Lonoke County." In a subsequent conversation Mr. Millar stated that he had not, at the time of collection, observed proliferations on plants growing on drier situations. In the modified spikelets examined either one or two florets were affected, the distal one of which was borne upon a much elongated rachilla which frequently reached 2 cm. in length and at times exceeded the somewhat enlarged subtending glumes (fig. 5).

Herbarium material.—Oat fields, Lonoke, Lonoke Co., Arkansas: *P. H. Millar* (May, 1939).

PANICUM VIRGATUM L.—During the three winters of 1937-1940 a number of plants of several selections of this species were grown in the greenhouse at this station. Some plants of selection no. 644 developed proliferations that entirely replaced inflorescences. These plants, grown from caryopses taken from plants growing along a railroad embankment a few miles north of Fayetteville, were planted in the greenhouse in early September, 1937. Growth of the seedlings appeared normal until after March 1, 1938, when the development of proliferation was first noted. By mid-April a few proliferations had reached the stage shown in figure 6. Some had produced several tillers, and adventitious roots had appeared on the short rhizomes. Plants of this no. 644 and of two other selections that have also proliferated under greenhouse conditions, no. 339 from O'Neil, Nebraska, and no. 1602 from Stillwater, Oklahoma, have since flowered normally and produced good yields of viable caryopses when grown under field conditions at the Experimental Farm, Fayetteville. On September 7, 1940, numerous proliferating plants of this species were observed in a pasture area. In this case the abnormalities developed only on those plants that had been retarded by the removal of their crowns through grazing.

A cytological examination of material taken from these selections of *Panicum* indicates no apparent reason to assume that in this species, at least, the vegetative mode of reproduction is necessarily associated with higher chromosome numbers (4, 5).

Herbarium material (taken from greenhouse-grown plants).—*Nielsen*, selection no. 644 (April, 1938, and March, 1939); no. 1602 (Jan. 9 and April 15, 1940); no. 339 (April 1, 1940). Northwest Arkansas Land Use Project, near Wedington, Arkansas: *Younge and Nielsen*, no. 6467.

Discussion

From the preceding observations and from many of those recorded in the cited bibliographies, it appears that adverse environmental factors, or abrupt changes of environmental factors—particularly moisture, light, and perhaps temperature—play an important part in the mode of reproduction of some of the numerous races of certain grass species. As indicated in the cited papers, form names have been applied in some instances to races showing tendencies toward proliferation. It seems advisable that such taxonomic description should be postponed until the physiological responses of a number of races within a given grass species have been studied under a wide range of controlled environmental conditions and the effects of such conditions upon mode of reproduction critically observed.

Summary

Proliferation has been observed to occur in *Festuca obtusa*, *Bromus inermis*, *B. purgans*, *Phleum pratense*, *Avena sativa*, and *Panicum virgatum*. The general external morphology of the proliferations is briefly described and the environmental conditions of the proliferating plants indicated.

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LITERATURE CITED

1. ARBER, AGNES, The Gramineae. A study of cereal, bamboo, and grass. New York. 1934.
2. HALPERIN, M., The taxonomy and morphology of bulbous bluegrass, *Poa bulbosa vivipara*. Jour. Amer. Soc. Agron. 25:408-414. 1933.
3. TURESSON, G., Studien über *Festuca ovina* L. I. Normalgeschlechtliche, halb und ganzvivipare Typen nordischer Herkunft. Hereditas 8:161-206. 1926.
4. ———, Studien über *Festuca ovina* L. II. Chromosomenzahl und Viviparie. Hereditas 13: 177-184. 1929-30.
5. ———, Studien über *Festuca ovina* L. III. Weitere Beiträge zur Kenntnis der Chromosomenzahlen viviparer Formen. Hereditas 15:13-16. 1931.

STRUCTURE OF STEMS IN RELATION TO DIFFERENTIATION AND ABORTION OF BLOSSOM BUDS¹

B. ESTHER STRUCKMEYER

(WITH THIRTY-EIGHT FIGURES)

Introduction

It was shown by WILTON and ROBERTS (6) that differences exist in the anatomical structure of the stems of nonflowering and flowering plants. WILTON (7) later reported that the degree of cambial activity in annual plants depends upon whether they are vegetative, budding, or flowering. She observed that the cambium becomes less active as the plants form blossom buds, and by the time these buds have developed into flowers and fruits, cambial activity has ceased. The characteristics of the phloem tissue in stems of nonflowering and flowering plants were described by STRUCKMEYER and ROBERTS (5). They found the sieve tubes and companion cells to be fewer in number, thicker walled, and smaller in size in stems of flowering than in nonflowering plants. Callose was also more abundant in stems of a number of species of flowering plants.

In connection with these investigations, the following questions arose: (a) Do the anatomical changes in stems of plants placed in an environment favorable for flowering become apparent before the appearance of blossom primordia, or at the same time as blossom primordia, or are these changes unrelated to the differentiation of blossom primordia? (b) What is the effect of return to an environment favorable to vegetative growth upon plants previously subjected to an environment inductive to flowering?

Material and methods

The plants were grown in the greenhouses of the department of horticulture at the University of Wisconsin. The five species under observation were:

Compositae: *Cosmos sulphureus* Cav. var. Klondike, *Xanthium echinatum* Murr.

Cruciferae: *Mathiola incana* R. Br. var. Christmas Pink

Labiatae: *Salvia splendens* Ker. var. Harbinger

Leguminosae: *Glycine max* Merr. var. Biloxi

Several series, each composed of approximately seventy plants of these species, were placed in greenhouses with minimum temperatures of 55°, 65°, and 75° F., and are respectively designated as cool, medium, and warm. Long-day conditions

¹ Published with the approval of the Director of the Agricultural Experiment Station. This investigation was supported in part by a grant from the Wisconsin Alumni Research Foundation.

were secured by the use of electric lights employed from about sunset until midnight. These delivered to the plants 30–80 foot candles, as measured by a Weston photometer. The normal winter days of November, December, and January were used as short days. Before November and after January, the days were artificially shortened to $9\frac{1}{2}$ –10 hours. Some of the plants placed on the short days were sampled at regular intervals while the remainder were returned to long days. The latter were also sampled after varying lengths of exposure.

Samples were taken of the fourth internode and of the stem tip of the same plants, three plants being taken at each sample date. The material was fixed in formalin-acetic-alcohol. Butyl alcohol was used as the dehydrating agent, and the material was imbedded in paraffin. Transverse and longitudinal sections 12–15 μ thick were cut. The sections were stained with 50 per cent alcoholic safranin and with light green in clove oil.

Observations

Plants requiring different environmental treatment for the formation of blossom buds were examined. Soybean, *Salvia*, *Cosmos*, and *Xanthium* initiate blossom buds when exposed to short days at appropriate temperatures (1, 2, 3, 4). Stock produces blossoms under long-day conditions at a cool and medium temperature, and after a greater length of time on short days at a cool temperature.

Microscopic blossom primordia were evident on plants of soybean and *Salvia* when exposed to 9 short days and a warm temperature. Unless this short-day treatment was continued approximately 17 days, the primordia failed to develop into flowers and the plants gradually acquired vegetative characteristics. Blossom primordia were apparent on plants of *Cosmos* given 12 short days, and an exposure of at least 18 short days was necessary for these plants to retain their blossom buds and continue development if transferred to long days. The time necessary for development of blossom primordia is greatly dependent upon the temperature. Stock requires even more time for the formation of blossom primordia, for only after 18 days of long-day treatment at a cool temperature were microscopic primordia apparent. At the warm temperature, these plants remained vegetative on both long and short days. On the other hand, primordia were apparent on plants of *Xanthium* after 5 days of short-day treatment at a warm temperature.

These results indicate that under the most favorable environmental conditions microscopic blossom primordia are apparent only after varying lengths of treatment for different species of plants. Perhaps more important is the fact that unless this favorable environmental treatment is continued, even after blossom primordia are formed, they will not develop into blossoms but will abort. *Xanthium*, however, does not exhibit this condition. Two to three short days are sufficient

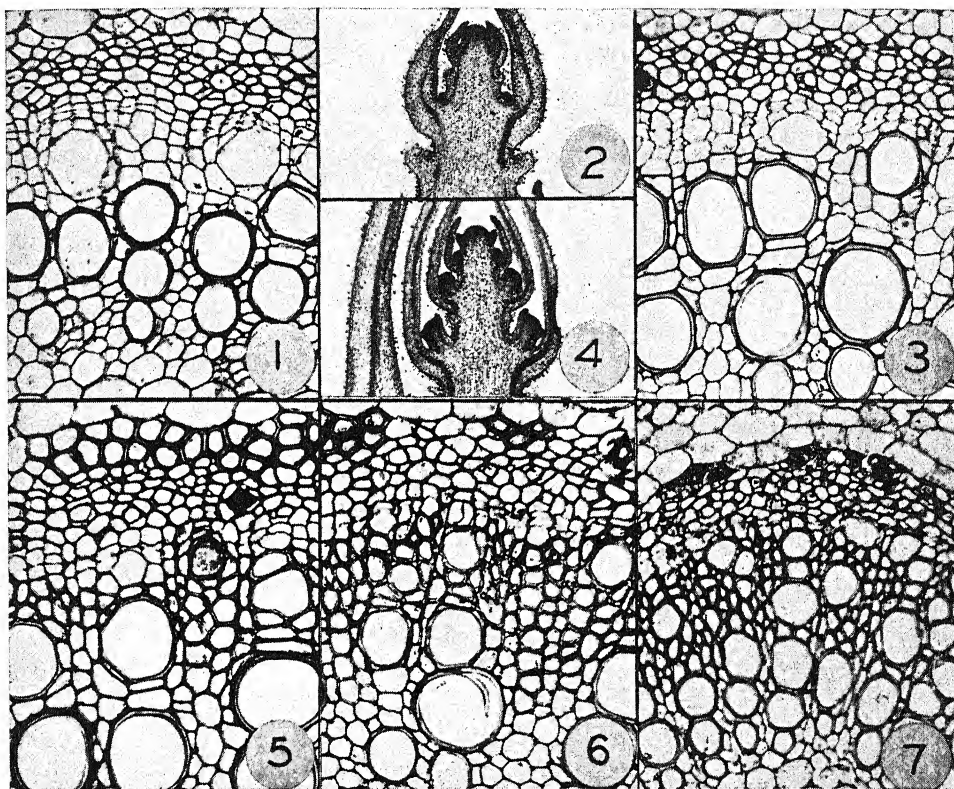
to induce this species to produce blossoms, even though the plants have been transferred to an environment favorable to vegetative growth. Staminate blossoms may be formed after plants are exposed to but one short day. Therefore soybean, *Salvia*, *Cosmos*, and stock require a certain period of exposure to a favorable environment for the initiation of blossom primordia, and this period must be continued for a length of time after primordia are formed if they are to develop into flowers.

The first question considered in this study is: When do the anatomical changes in the stem first become apparent in plants placed in an environment favorable to flowering? During this investigation it was observed that the transformations in anatomical structure were apparent soon after the beginning of a treatment inductive to flowering. *Salvia* illustrates this condition. Stems of plants given 5 short days displayed a less active cambium than plants that remained on long days at a warm temperature. The former also showed a decrease in the differentiation of number of xylem vessels, sieve tubes, and companion cells (figs. 1-3). Although alterations in the structure of the stem were apparent after 5 short days, microscopic blossom buds were not evident until 8-9 short days. Plants given 8 short days showed a further reduction in meristematic tissue. Little differentiation of tissue was in progress at this stage, although a number of the cells were becoming thicker walled (figs. 4, 5). In stems of plants given additional short days, the cambial region—which at this time was limited to one or two layers of cells—continued to give rise to xylem cells. Few if any phloem cells were being formed and these were composed chiefly of phloem parenchyma cells. Continued thickening of the walls of the vascular tissue and the pericycle was apparent (fig. 6). The stem tips showed progressive differentiation of the primordia into blossom buds. The stems of plants that had received 39 short days showed no active cambium. The sieve tubes and companion cells were smaller than those in the stems previously described (fig. 7). By this time the plant was in flower.

Similar changes in anatomical structure accompanying blossom initiation were observed for soybean, in that alterations in the structure of the stem were apparent after 6 short days while blossom primordia were not apparent until 9-12 short days at a warm temperature (figs. 8-12). Likewise for soybean, the differences in anatomical structure became more pronounced as the plants were given additional short days. Plants given 39 short days, and which by this time had macroscopic blossom buds, showed the absence of a cambium in the stem. There was no evidence of meristematic tissue and the cells had become increasingly thicker walled (figs. 14, 15).

The alteration of the anatomical structure in *Cosmos* closely resembles that already described for *Salvia* and soybean (figs. 18-22). *Xanthium* presents a similar condition. It responds more quickly to photoperiod at the proper temperature

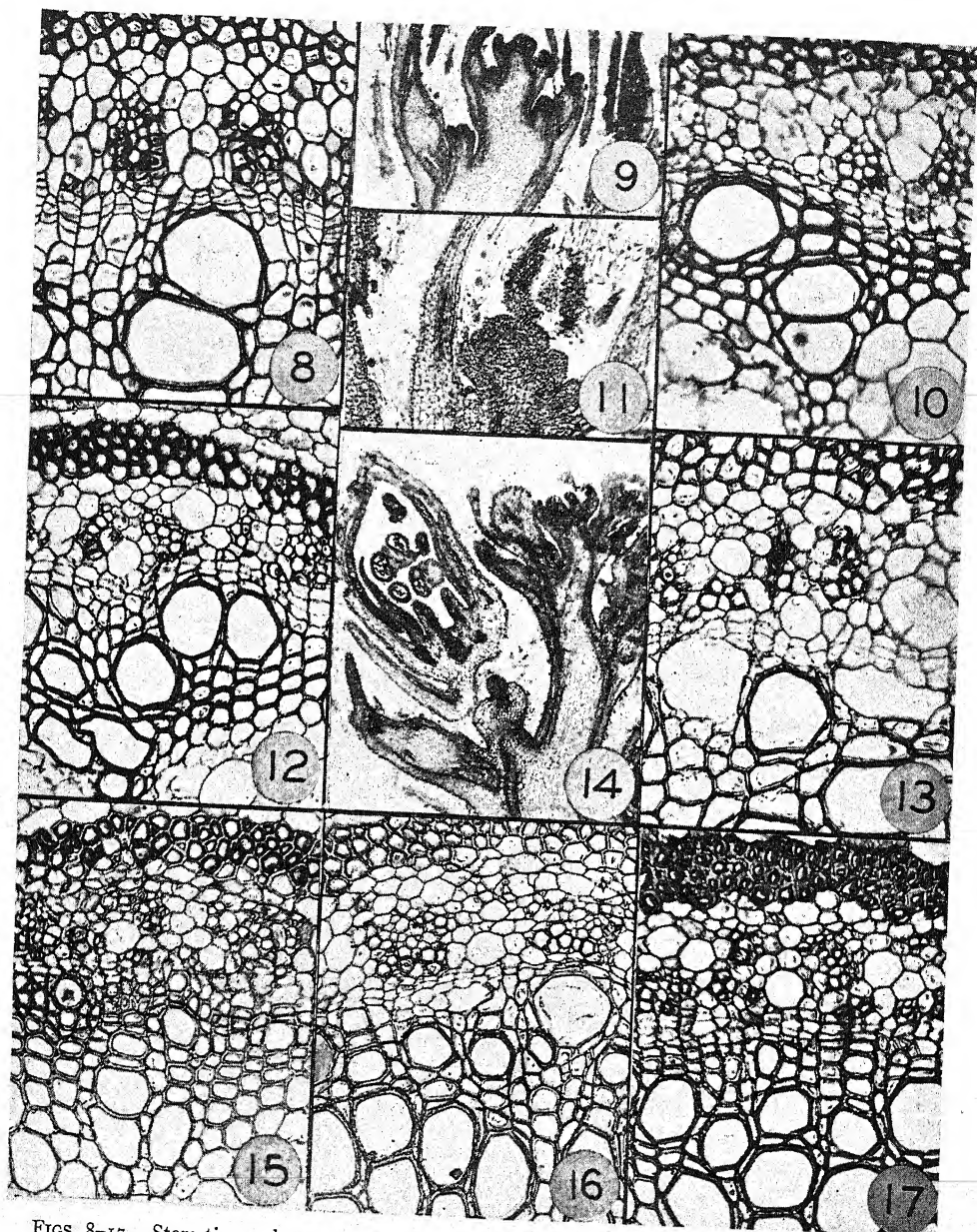
than do the other species studied. At a warm temperature, 2-3 short days are sufficient to induce this species to flower. Although blossom primordia are not present after 3 short days, they are evident on plants that have received 4-5 short days or 2 short days and 3-4 long days. Plants given 3 short days showed no distinguishable blossom primordia, but the structure of the stem had become altered



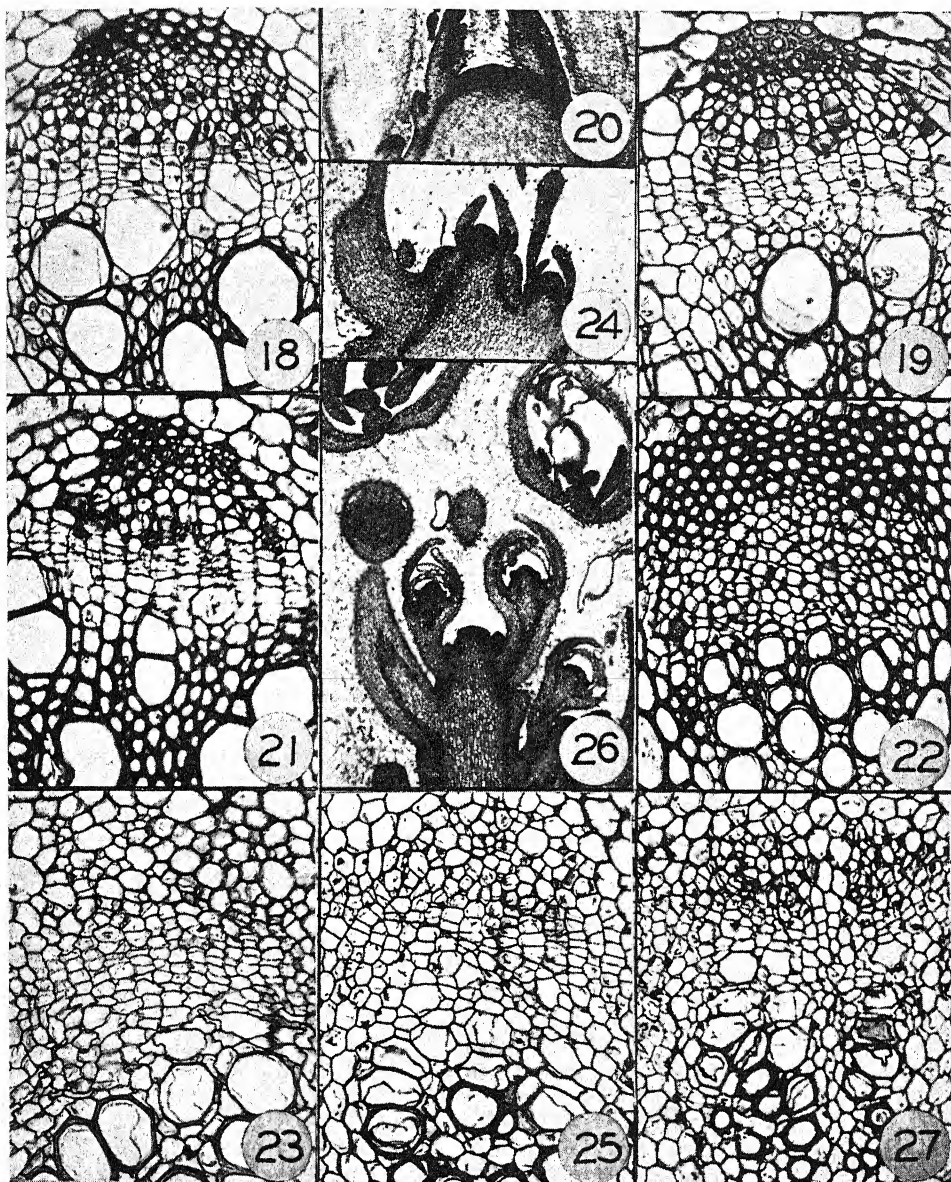
FIGS. 1-7.—Stem tips and transections of fourth internode of *Salvia*: Fig. 1, nonflowering plant; active cambium present. Figs. 2, 3, plant exposed to 5 short days; no apparent blossom primordia, less active cambial region, fewer xylem vessels differentiating, and phloem parenchyma rather than sieve tubes forming. Figs. 4, 5, 8 short days; prominent blossom primordia, cambium less active. Fig. 6, 22 short days; cells of stem thick walled, formation of vascular tissue almost ceased. Fig. 7, flowering plant; cells of stem small and thick walled, little or no meristematic tissue present.

(figs. 28-31). A less active cambial zone was apparent, fewer secondary elements were being formed, and the cells surrounding the xylem vessels were becoming thick walled. As the primordia developed into blossoms the cambium remained somewhat active, giving rise to new xylem vessels (figs. 32, 33).

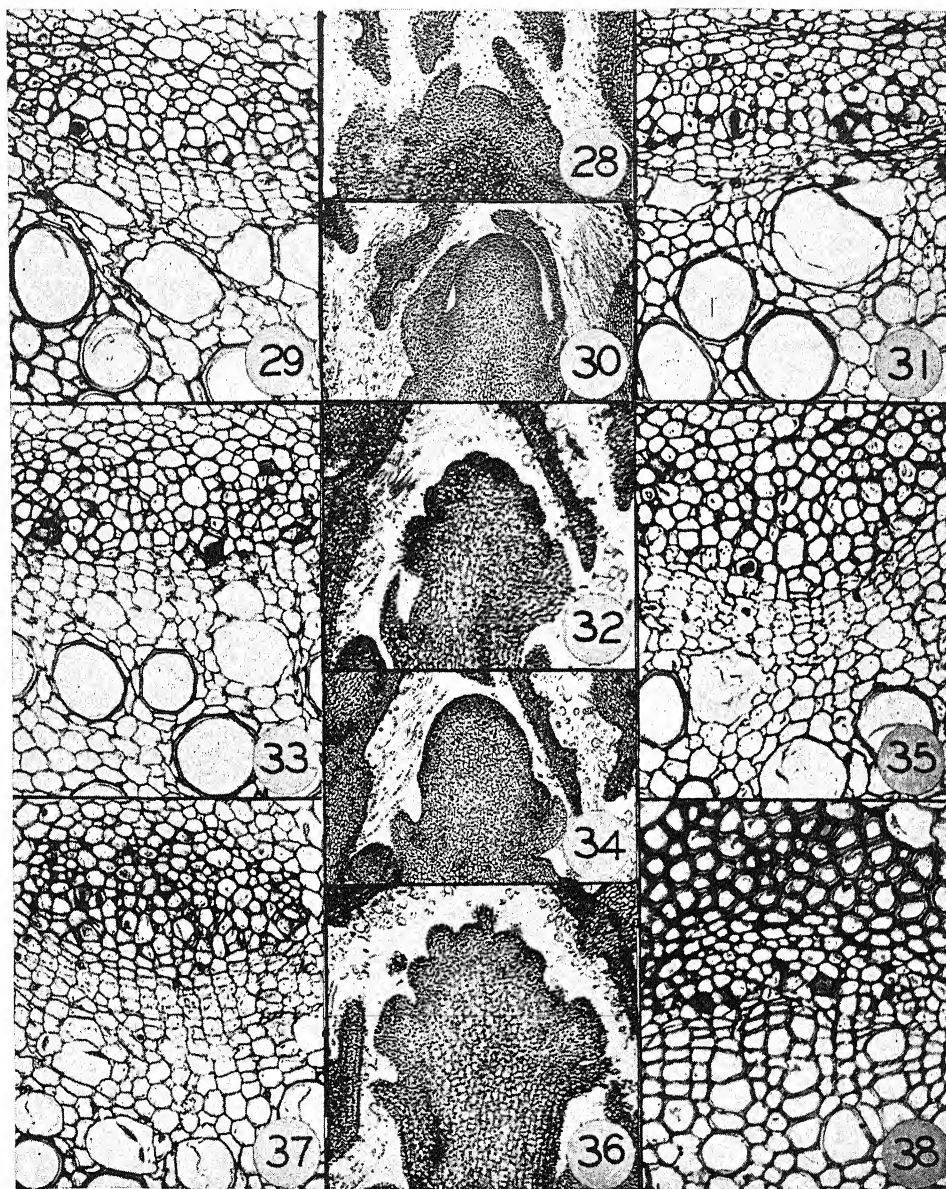
Cool temperature delayed the flowering of *Xanthium* on short days. Plants



FIGS. 8-17.—Stem tips and transections of fourth internode of soybean: Fig. 8, nonflowering plant; active cambium producing vascular tissue. Figs. 9, 10, 6 short days; no blossom primordia apparent, less active cambium, fewer xylem vessels, and phloem cells in process of differentiation. Figs. 11 (tip of axillary), 12, 12 short days; blossom primordia present, fewer meristematic and developing cells. Fig. 13, 5 short and 7 long days; 7 long days sufficient time for stem again to acquire characteristics of vegetative stem; cambium active. Figs. 14, 15, 39 short days; blossom buds present, meristematic tissue apparently absent in this internode. Fig. 16, 17 short and 22 long days; some cambial activity; plants given this treatment usually again acquire vegetative characteristics. Fig. 17, 24 short and 15 long days; cells of stem have become mature.



FIGS. 18-27.—Stem tips and transections of fourth internodes of stems. Figs. 18-22, *Cosmos*: Fig. 18, nonflowering plant; active cambium forming vascular tissue. Fig. 19, 3 short days; cambium somewhat less active than in vegetative stem. Figs. 20, 21, 12 short days; blossom primordia apparent, stem shows reduced cambial activity. Fig. 22, flowering plant; stem has no apparent meristematic tissue. Figs. 23-27, stock: Fig. 23, nonflowering plant; vegetative stem possesses features already described for other vegetative stems. Figs. 24, 25, 6 long cool days; no primordia apparent, slightly less active cambium. Figs. 26, 27, 30 long cool days; blossom buds conspicuous, stem possesses relatively inactive cambium.



FIGS. 28-38.—Stem tips and transections of fourth internode of *Xanthium*: Figs. 28, 29, nonflowering plant; cambium composed of several layers of meristematic cells; xylem vessels and phloem cells differentiating. Figs. 30, 31, 3 warm short days; no blossom primordia apparent, less active cambium. Figs. 32, 33, 7 short days; blossom primordia evident, reduction in amount of meristematic tissue. Figs. 34, 35, 9 cool short days; plant comparable in development to one given 3 warm short days. Figs. 36, 37, 20 cool short days; cool temperature delayed budding; structure of stem and tip comparable in development with the one given 7 warm short days. Fig. 38, fruiting plant; no apparent meristematic tissue, cells have become thick walled.

given 9 short days at a cool temperature were comparable in development with those given 3 short days at a warm temperature, and plants given 20 short days at a cool temperature possessed blossom primordia comparable with those given 7 short days at a warm temperature (figs. 34-38).

Stock is classified as a long-day plant. When grown at a cool temperature, however, it also blossoms on short days. It requires approximately 18 long days for blossom primordia to appear when grown at a medium temperature. In the long-day plants, differences in anatomical structure were apparent prior to the appearance of blossom primordia. Twenty-four and 30 days of long-day treatment resulted in the differentiation of blossom buds and further reduction in meristematic tissue (figs. 23-27).

From these observations it is evident that alterations in the vascular structure occur relatively early in the development of plants exposed to an environment favorable to flowering. What the significance of this might be is not yet known, but it is evident from the species examined that although different environmental conditions must be used to induce flowering, yet a similar internal structural development of the stem occurs.

The second question considered is: What is the effect of an environment favorable for vegetative growth upon plants previously exposed to an environment inductive to flowering? The vascular structure of the stems of those plants that had formed blossom primordia but which aborted when the plants were transferred to an environment favorable for vegetative growth was examined. Provided the exposure to the environment inductive to flowering had not brought about sufficient changes in anatomical structure, the cambium resumed its activity and differentiated new xylem and phloem elements. Soybean illustrates this condition. Plants given 12 short days possessed blossom primordia. Cross sections of stems indicated that the cambium was less active than in the vegetative stem, and the walls of the cells were becoming thickened. Phloem parenchyma instead of sieve tubes and companion cells were being formed. When comparing stems of plants given 12 short days with those that had received 12 days of treatment, 5 of which were short and 7 of which were long, it was found that the primordia were not yet apparent in the latter. The cambium in the stems of these plants was active, and vascular tissue was being differentiated (fig. 13). Twelve short days and 6 long days resulted in subsequent abortion of the blossom primordia and return of the plant to the vegetative condition. Another group of plants was given 39 days of environmental treatment. Some of these plants were exposed to a number of short days and then transferred to long days. Plants given 17 or more short days previous to their transfer to long days possessed blossom buds that matured into flowers and fruits. These plants had stems with little or no apparent meristematic tissue (figs. 16, 17).

Twelve long days followed by 12 short days were not favorable for producing blossom primordia in plants of stock.

When flowering plants of soybean, *Salvia*, *Cosmos*, and stock are desired, therefore, it is necessary to expose them to the environment inductive to flowering for a greater length of time than just the induction period required for the apparent changes in anatomical structure and the formation of blossom primordia. It is not sufficient to secure only primordia, for unless the environment is favorable for development of blossoms, abortion will occur.

The effects which the observed changes in anatomical structure prior to blossoming may have upon physiological relations have not yet been studied. The fact that comparable structural changes occur in plants at the time of blossom induction, without regard to the environmental conditions used to initiate the blossom state, would seem an added means of studying the nature of blossoming in flowering plants.

Summary

1. Soybean, *Salvia*, *Cosmos*, and *Xanthium*, all short-day plants, initiate blossom primordia after different durations of exposure to short days. Soybean and *Salvia* require 9, *Cosmos* 12, and *Xanthium* 5 days of short-day treatment for the appearance of blossom primordia at the appropriate temperature and cultural conditions. Stock, a long-day plant (at a medium or cool temperature), requires approximately 18 days for the appearance of primordia.

2. In order that the blossom primordia may continue to develop, it is necessary to expose the plants studied (except *Xanthium*) to a favorable environment for a greater length of time than just the induction period. *Xanthium* requires an induction period of only 2-3 short days at a warm temperature for the initiation and development of blossom buds. One short day is sufficient for the initiation of staminate blossoms.

3. Alterations in the anatomical structure of the stem occurred early in the development of plants placed in an environment favorable to the formation of blossom primordia. The first indication of a change in structure was a decrease in the number of meristematic cells of the cambial zone. Associated with this was the simultaneous decrease in the formation of xylem and phloem cells and a thickening of the cell walls of the vascular tissue. The cambium generally remained somewhat active, giving rise to new xylem but little phloem. The most recently formed phloem was composed almost entirely of phloem parenchyma cells. The stems of flowering and fruiting plants had little or no meristematic tissue, and the walls of the cells had become greatly thickened.

4. Plants of soybean, *Salvia*, *Cosmos*, and stock exposed to an environment favorable to flowering for a limited time only, and then returned to conditions

inductive to vegetative growth, soon showed renewed cambial activity and differentiation of vascular elements with the failure of the blossom primordia to continue development. Plants of *Xanthium* given 2-3 short days became reproductive regardless of subsequent treatment.

The writer expresses her appreciation to Professors R. H. ROBERTS and EMMA L. FISK for valued suggestions and constructive criticism generously contributed throughout the progress of this investigation.

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LITERATURE CITED

1. MANN, L. K., Effect of some environmental factors on floral initiation in *Xanthium*. BOT. GAZ. 102:339-356. 1940.
2. PARKER, M. W., and BORTHWICK, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. BOT. GAZ. 101: 145-167. 1939.
3. ROBERTS, R. H., and STRUCKMEYER, B. ESTHER, The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. Jour. Agr. Res. 56:633-677. 1938.
4. ———, Further studies of the effects of temperature and other environmental factors upon the photoperiodic responses of plants. Jour. Agr. Res. 59:699-709. 1939.
5. STRUCKMEYER, B. ESTHER, and ROBERTS, R. H., Phloem development and flowering. BOT. GAZ. 100:600-606. 1939.
6. WILTON, O. CHRISTINE, and ROBERTS, R. H., Anatomical structure of stems in relation to the production of flowers. BOT. GAZ. 98:45-64. 1936.
7. WILTON, O. CHRISTINE, The correlation of cambial activity with flowering and regeneration. BOT. GAZ. 99:854-864. 1938.

SIROCLADIUM, A NEW TERRESTRIAL MEMBER OF THE ZYGNEMALES

M. S. RANDHAWA

(WITH FIFTEEN FIGURES)

This alga was collected by the writer from the Kumaon Hills, at an altitude of about 5500 feet, in September, 1939. It was found on soft clay under an overhanging rock, at a distance of about 10 feet from a small waterfall, growing in the form of dark green feltlike radial patches about 1-2 cm. in diameter. Unlike some terrestrial species of *Vaucheria* and *Oedogonium terrestris*, it was never found in the form of mats. The moist clay on which it was growing received a light spray from the waterfall. The alga was shaded by an overhanging rock on the north and by the shade of a tree on the south, and was also protected by another rock, so that it never received direct sunlight.

Observations

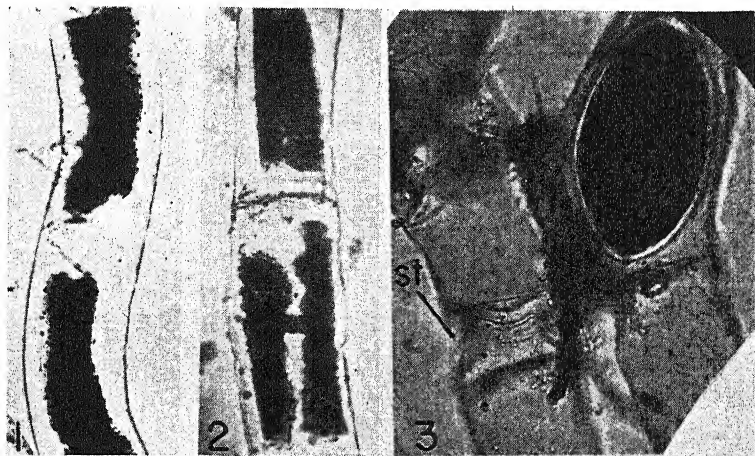
CHLOROPLASTS.—The cells are rectangular in outline, 45-64 μ broad and 120-210 μ long. Septa are plane. Most of the subaerial cells have two flat, axile, plate-shaped chloroplasts, each of which is as broad as the cells, after allowing for thickness of cell walls. These two chloroplasts lie parallel to each other laterally, and in most cases cover each other so completely that the cells seem to contain only a single chloroplast (figs. 1, 2, 6), and are indistinguishable from those of broad species of *Mougeotia*.

The margin of these platelike chloroplasts is usually irregular, and each bears a number—4-17 as a rule—of conspicuous pyrenoids, scattered irregularly (figs. 4-6). In some cases the pyrenoids are surrounded with rings of starch particles (fig. 4). The broad platelike chloroplasts completely fill the interior of the cells in fresh material, and considerable shrinkage takes place, even when preserved in 4 per cent formalin.

In cells close to the soil the chloroplasts are reduced in size and are 20-30 μ broad; that is, approximately half the size of the cells. When seen edgewise these chloroplasts appear as linear, threadlike bodies. In some cases these narrow plates overlap in such a manner that they simulate a single plate and their double nature is seen only by a change of focus under the high power. As one follows the cells into the soil, a gradual reduction is noticed in the size of the two platelike chloroplasts, which are ultimately reduced to two linear threadlike chloroplasts joined together by a cytoplasmic isthmus in which a pale blue conspicuous nucleus is

seen. Pyrenoids in these reduced chloroplasts are also considerably attenuated (figs. 10-15). Further down only fragments of chloroplasts remain in some cells, while the others are almost empty.

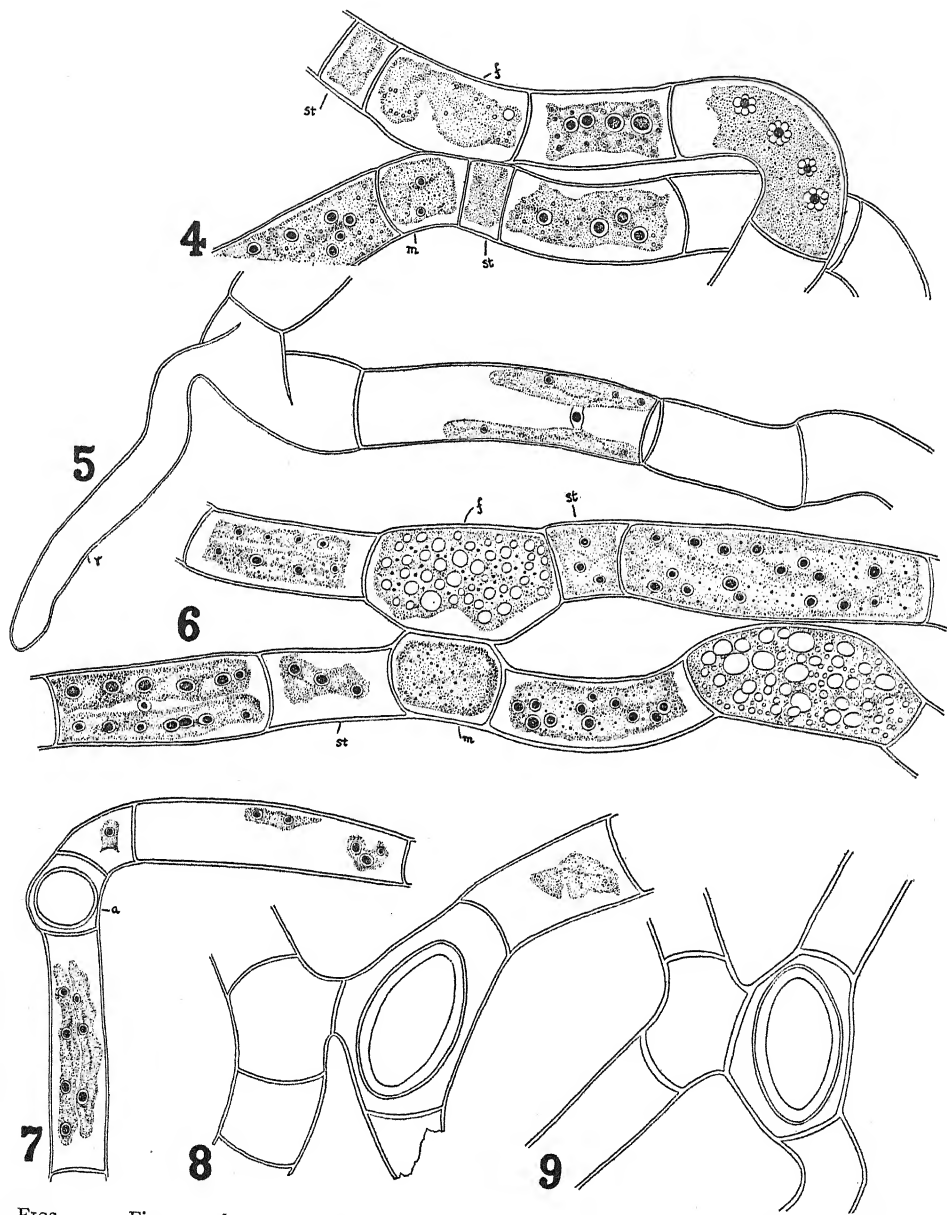
RHIZOIDS.—The subterranean cells are usually much elongated and are narrower as compared with the subaerial cells, as in most terrestrial algae. In some cases the terminal cells are drawn out into rhizoidal structures (figs. 12, 15), and occasionally even an intercalary cell gives out a rhizoidal structure laterally (fig. 5). Some of the intercalary cells which happen to get buried under soil particles



FIGS. 1-3.—*Sirocladium kumaoensis* gen. et sp. nov. Figs. 1, 2, subaerial cells showing chloroplasts. Considerable shrinkage of protoplasm and chloroplasts occurred in this preserved material. Fig. 3, ripe zygospore and sterile cell (*st*).

lengthen considerably and serve a rhizoidal function (figs. 13, 14). Even branching may be observed in some of the rhizoidal cells (fig. 10).

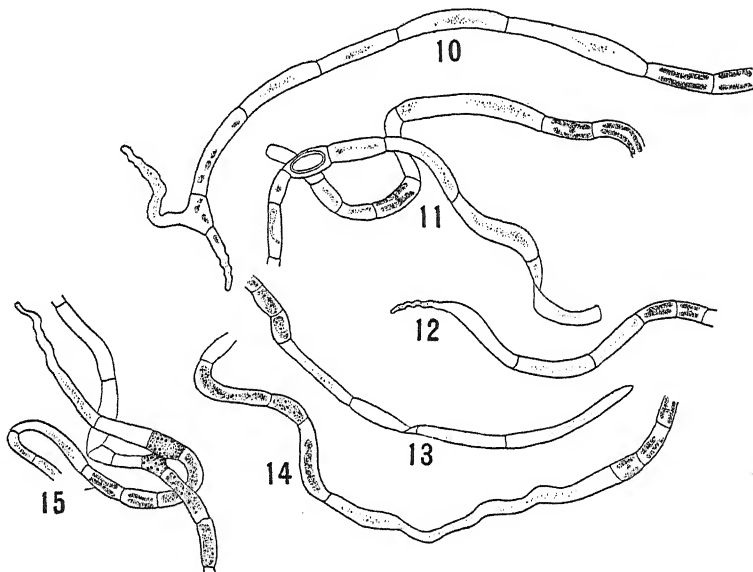
CONJUGATION.—The mode of conjugation in *Sirocladium* is similar to that of *Sirogonium*. Filaments in all stages of conjugation were collected on September 7, and mature zygospores were seen in abundance on September 23 and also October 17, the last collection. There is a noticeable difference in the size of male and female cells, and the latter are loaded with starch and other food materials. The filaments are usually much more coiled in conjugating than in sterile material, and probably this helps in the process by providing points of contact which stimulate tactile responses. As in *Sirogonium*, only a single sterile cell is cut off from the female cell, while one or two sterile cells are cut off from the cell which ultimately functions as the male (figs. 4, 6). Mucilaginous pads are seen at the point of contact between the mating cells (fig. 3). As in *Sirogonium*, conjugation is of a typically geniculate type, although in some cases lateral cell walls may be lengthened



FIGS. 4-9.—Fig. 4, early stage in conjugation; sterile cells (*st*) cut off from male (*m*) and female (*f*) cells. Fig. 5, lower part of filament showing attenuated chloroplasts and rhizoid (*r*). Fig. 6, later stage in conjugation showing increased size of male and female cells and different types of chloroplasts. Fig. 7, an azygospore (*a*). Figs. 8, 9, conjugation and ripe zygospores.

at the point of junction, simulating a tube (fig. 8). Zygospores are ellipsoid in shape, $42-70\mu$ broad and $100-118\mu$ long. Ripe zygospores are yellowish brown in color, with a smooth mesospore. Conjugation takes place not only among sub-aerial cells, but in some cases it was noticed that even underground cells conjugated and produced ripe zygospores. In such cases the conjugating cells as well as the neighboring ones do not contain chloroplasts, although they are somewhat rich in protoplasm (fig. 8).

AZYGOSPORES.—As in certain species of *Spirogyra* and *Zygnema*, the contents of the male cells in some instances develop into rounded azygospores (fig. 7). This



FIGS. 10-15.—Different types of rhizoids

may be due to the fact that after the formation of a male cell has been induced by contact with another filament, that filament is displaced and moves apart, owing to action of air or impact with drops of water.

Discussion

So far as the mode of conjugation is concerned, *Sirocladium* resembles *Sirogonium*; there is the same elimination of conjugation canals and inequality in the size of the conjugating cells. In the structure of its chloroplasts, however, this alga resembles certain broad species of *Mougeotia*, and in its habit, species of *Zygonium* and *Zygnema terrestris* (2). More minutely considered, the chloroplasts of *Sirocladium* are unique; while chloroplasts of *Mougeotia* are axile, in this alga the two broad plates are lateral in position.

Only a system of classification of Zygnemales which takes into consideration both the structure of chloroplasts as well as the modes of reproduction can be regarded as satisfactory (3). CZURDA (1) bases the genera of Zygnemales on the structure of chloroplasts alone, but this concept involves difficulties and inconsistencies. According to such a system it is not difficult to group together forms like *Zygnema*, *Zygogonium*, *Pleurodiscus*, and *Zygnemopsis* into an all-embracing genus *Zygnema*; and *Mougeotia*, *Debarya*, and *Temongametum* into *Mougeotia*; but an alga like the present one presents a problem. If CZURDA's system of classification is followed, the present form must be placed close to *Mougeotia*. If only the mode of reproduction is considered, widely diverse forms will be brought together; for example, species of both *Zygnema* and *Spirogyra* show anisogamous conjugation, which hardly justifies grouping them together in the same genus. Following the same principle, the mere presence of geniculate conjugation and absence of conjugation canals do not justify the inclusion of the present alga in genus *Sirogonium*. When the alga was originally collected, in a sterile condition in early September, the broad platelike chloroplasts of the subaerial cells indicated a terrestrial species of *Mougeotia*, and only when conjugating cells were found was its affinity with *Sirogonium* suggested. Moreover, no terrestrial species of *Sirogonium* is known so far, and platelike chloroplasts are not known in any species of *Sirogonium*. It is true that the reduced chloroplasts in some of the subterranean cells show resemblances with some species of *Sirogonium*, in which the number of chloroplasts is few, but even then the resemblance is superficial, for there is no known species of *Sirogonium* in which the number of chloroplasts is reduced to the fixed number of two. In nearly all the species of *Sirogonium* the number of chloroplasts is usually 5-8 or more, and only in a few cells are they reduced to one or two on account of certain physiological factors. Its peculiar terrestrial habitat, its varied chloroplasts, and its mode of conjugation, justify the formation of a new genus of the Zygnemales, which is named *Sirocladium*.

Sirocladium takes its place in the family Mougeotiaceae of the order Zygnemales, next to *Sirogonium*. So far as its terrestrial habit and presence of rhizoids is concerned, it is certainly a more advanced and specialized form than *Sirogonium*. It is futile to make any generalization about the comparative primitiveness or advanced nature of axile platelike chloroplasts as compared with semi-spiral or straight bandlike chloroplasts, for each represents an adaptation toward particular environmental conditions.

Sirocladium gen. nov.

Filaments usually simple with lowest cells drawn out into rhizoids, rarely with lateral branches of a few cells in the subterranean part. Cells cylindrical with plane septa, each containing two lateral plate-shaped chloroplasts which in cells

close to the soil or partly subterranean are reduced to two bandlike wavy bodies. Conjugation direct between genuflexed cells, without the intermediation of conjugation tubes. One or more sterile cells cut off from both female and male cells, the former considerably larger than the latter. Zygosporcs ellipsoid. The only recorded species is terrestrial in habit.

Sirocladium kumaoensis sp. nov.

Characters as those of the genus.

Vegetative cells $45-64\mu$ broad and $120-210\mu$ long; fertile cells inflated up to 90μ in diameter; zygosporcs ellipsoid, $42-68\mu$ diameter, $102-118\mu$ long; spore wall smooth, yellowish brown.

Growing on moist clay under a shady rock in Kumaon Hills, Himalayas, Almora U.P., India, in September and October, 1939.

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LITERATURE CITED

1. CZURDA, V., Zygnemales in Die Susswasserflora Mitteleuropas. Heft 9. 1932.
2. RANDHAWA, M. S., Observations on some Zygnemales from northern India. Proc. Ind. Acad. Sci. 8:Sec. B. 1938.
3. TRANSEAU, E. N., The genera of the Zygnemataceae. Trans. Amer. Micros. Soc. 53:201. 1934.

CHROMOSOMES OF SOME ALISMACEAE

ELEANOR MARIE OLESON

(WITH NINE FIGURES)

Introduction

LIEHR (1) determined the diploid chromosome number of *Alisma plantago* L. as 12. WULFF (10) reported it as 10, but was uncertain as to the accuracy of his count since the chromosomes are long and twisted.

LIEHR (1) reported 16 as the diploid number in *Sagittaria sagittifolia* L. LOHAMMAR (2), however, found 22 in this species, as well as in *S. natans* Pallas. According to LOHAMMAR, there is little cytological difference between the two species; while the chromosomes differ somewhat in size, the idiograms of the two are similar. In both species there are two long chromosomes with central fiber-attachment constrictions. One, possibly two, have primary constrictions at approximately one-third the distance from one end. The other chromosomes have subterminal constrictions. In the metaphases they are long and twisted. TAYLOR (8) reported 20 as the apparent diploid number in *S. montevidensis* Cham. and Schlecht. but found difficulty in counting the long twisted chromosomes. In two, possibly four, of the chromosomes the primary constrictions are median; in the others they are subterminal.

NAWA (5) found 20 to be the diploid number in *S. trifolia* var. *sinensis* (Sims) Makino. For the same species, MORINAGA and FUKUSHIMA (3) reported 11 as the haploid number and 22 as the diploid. According to the latter investigators there are 2 long chromosomes with median fiber attachments; 2 have subterminal and 18 have terminal fiber attachments. In *S. aginashi* Makino, SHINKE (6, 7) determined the haploid number as 11, the diploid as 22. The chromosomes fall roughly into four groups with respect to size and position of fiber-attachment points. Two long chromosomes have central fiber-attachment constrictions. Two very short chromosomes are rod-shaped, the position of the fiber-attachment points not being designated. The remaining 18 are rod-shaped with subterminal primary constrictions. They are divided into two groups; in one the constrictions are clear and deep, in the other group they are obscure. The internal spiral structure of the meiotic chromosomes was studied in detail. NARASIMHA MURTHY (4) found the haploid number in *Limnophyton obtusifolium* (L.) Miq. to be 12.

Table 1 gives the chromosome numbers reported for members of this family, including the results of the present study.

Material and methods

During the summer months of 1939, root tips and buds of *Alisma plantago* L., *Sagittaria latifolia* Willd., and *S. rigida* Pursh. were taken from plants growing in marshes in Columbia County, Wisconsin, and immediately fixed, using Flemming's medium solution and Belling's and Randolph's modifications of Navashin's formula. Sections were cut at 10 μ .

Material fixed in the Flemming solution was stained with Heidenhain's iron-alum haematoxylin. The crystal violet-iodine stain followed by picric acid was used with a portion of the material fixed in Belling's and Randolph's solutions.

TABLE 1

SPECIES	<i>n</i>	<i>2n</i>	INVESTIGATOR
<i>Alisma plantago</i>		12	Liehr, 1916
<i>A. plantago</i>		10	Wulff, 1939
<i>A. plantago</i>		14	Oleson
<i>Limnophyton obtusifolium</i>	12	Narasimha Murthy, 1933
<i>Sagittaria aginashi</i>	11	22	Shinke, 1929, 1934
<i>S. latifolia</i>	11	22	Oleson
<i>S. montevidensis</i>		20	Taylor, 1925
<i>S. natans</i>		22	Lohammar, 1931
<i>S. rigida</i>		22	Oleson
<i>S. sagittifolia</i>		16	Liehr, 1916
<i>S. sagittifolia</i>		22	Lohammar, 1931
<i>S. trifolia</i> var. <i>sinensis</i>		20	Nawa, 1928
<i>S. trifolia</i> var. <i>sinensis</i>	11	22	Morinaga and Fukushima, 1931

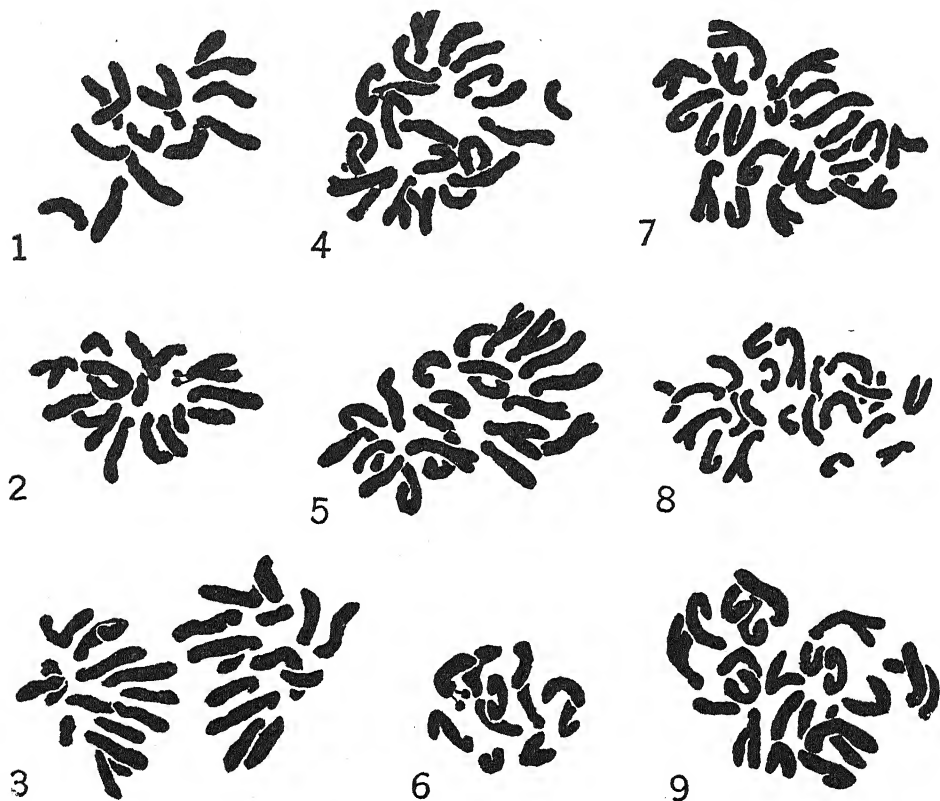
La Cour's crystal violet-iodine schedule was used for the remainder of the material. Material fixed in Belling's solution and stained by La Cour's method was found most satisfactory for the study of chromosomes.

Observations

The somatic chromosome number in *Alisma plantago* (figs. 1-3) is 14. This number does not agree with that reported either by LIEHR ($2n = 12$) or by WULFF ($2n = 10$). For this reason the count here given was decided upon only after a considerable number of favorable equatorial plates and anaphase figures were examined and drawn.

The chromosomes are long and twisted in equatorial-plate figures. They may be classed as long, medium, and short. Primary constrictions are of two types, median and subterminal. Four chromosomes are long, 2 having median and 2 subterminal constrictions. The remaining 10 have subterminal constrictions; 6 of these are of medium length and 4 are short. The primary constrictions are conspicuous in most cases. Secondary constrictions are generally obscure and were observed in only a few instances. In one equatorial plate (fig. 2) an already

divided satellite is attached to the short arm of one of the short chromosomes. The length of the attaching fiber equals approximately the diameter of the chromosome body. The homologous chromosome can be identified, but it is in a crowded position and the presence of a satellite cannot be determined.



FIGS. 1-9.*—Fig. 1, *Alisma plantago*, equatorial plate. Fig. 2, same showing one short chromosome with divided satellite. Fig. 3, same showing anaphase with 14 chromosomes in each group. Figs. 4, 5, *Sagittaria latifolia*, equatorial plate. Fig. 6, same, microspore. Metaphase showing one chromosome with divided satellite. Figs. 7, 8, 9, *S. rigida*, equatorial plate.

* Drawings made with camera lucida, using Spencer 1.5 mm. objective and compensation ocular 12X. All except fig. 6 are from cells of root tips. X 2200.

The haploid chromosome number in *Sagittaria latifolia* (fig. 6), determined from division figures in microspores, is 11. Five chromosomes are long, 4 of medium length, and 2 short. Primary constrictions are commonly obscure. Two long chromosomes have median constrictions; in the others the constrictions are subterminal. One short chromosome has been seen to have a rather large divided satellite attached to the short arm (fig. 6).

Twenty-two chromosomes appear during mitosis in root-tip cells (figs. 4, 5). They are long and twisted in equatorial plates. Ten chromosomes are long, 8 of medium length, and 4 short. Primary constrictions are usually conspicuous and deep. Four of the long chromosomes have median constrictions; the other 18 have subterminal constrictions. Secondary constrictions are visible in only a few cases. Satellites were not observed in somatic cells.

In *Sagittaria rigida* (figs. 7-9) 22 chromosomes are present in somatic cells. The chromosomes are of approximately the same size and the same general appearance as in *S. latifolia*. The primary constrictions are commonly distinct. Ten chromosomes are long, 2 having median and 8 subterminal constrictions. Eight chromosomes are of medium length, 2 having median and 6 subterminal constrictions. There are 4 short chromosomes with subterminal constrictions. Secondary constrictions appear in the long arms of a few chromosomes with subterminal primary constrictions. Satellites were not observed.

It is suggested that the basic chromosome numbers of the Alismaceae thus far studied are 7 in *Alisma*, 12 in *Limnophyton*, and 11 in *Sagittaria*. Although diploid numbers of 16 and 20 have been reported for certain species of *Sagittaria*, and 12 and 10 by previous observers for *A. plantago*, there is some uncertainty as to the accuracy of these numbers. My observations on the last-named species seem to indicate that previous counts were erroneous, unless varieties occur with different numbers.

Summary

1. The diploid chromosome number in *Alisma plantago* is 14. In *Sagittaria latifolia* the haploid chromosome number is 11, the diploid number 22. The diploid chromosome number in *S. rigida* is 22.
2. The chromosomes of these three species are similar in size and appearance. Primary constrictions are of two types, median and subterminal. The chromosomes may be distinguished as long, medium, and short.
3. Satellite chromosomes of *A. plantago* and *S. latifolia* show the same general characteristics as to length of chromosome body, position of the primary constriction, and size of satellites.
4. Basic chromosome numbers are probably 7 in *Alisma*, 12 in *Limnophyton*, and 11 in *Sagittaria*.

The writer is indebted to Dr. C. E. ALLEN, under whose direction this work was done.

LITERATURE CITED

1. LIEHR, L. O., Ist die angenommene Verwandtschaft der Helobiae und Polycarpicae auch in ihrer Cytologie zu erkennen? Beitr. Biol. Pflanz. 13:135-220. 1916.
2. LOHAMMAR, G., The chromosome numbers of *Sagittaria natans* Pallas and *S. sagittifolia* L. Svensk. Bot. Tidskr. 25:32-35. 1931.
3. MORINAGA, T., and FUKUSHIMA, E., Chromosome numbers of cultivated plants. III. Bot. Mag. Tokyo 45:140-145. 1931.
4. NARASIMHA MURTHY, S. K., Half-yearly Jour. Mysore Univ. 7:1. 1933. (Cited by TISCHLER, 9)
5. NAWA, N., Some cytological observations in *Tricyrtis*, *Sagittaria*, and *Lilium*. Bot. Mag. Tokyo 42:33-36. 1928.
6. SHINKE, N., Chromosome arrangement. IV. The meiotic divisions in pollen mother cells of *Sagittaria aginashi* Makino and *Lythrum salivaris* L. var. *vulgare* D.C. subvar. *genuina* Koehne. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. 4:283-308. 1929.
7. ———, Spiral construction of chromosomes in meiosis in *Sagittaria aginashi*. Mem. Coll. Sci. Kyoto Imp. Univ. Ser. B. 9:366-392. 1934.
8. TAYLOR, W. R., Chromosome constrictions as distinguishing characteristics in plants. Amer. Jour. Bot. 12:238-244. 1925.
9. TISCHLER, G., Pflanzliche Chromosomen-Zahlen. Tab. Biol. 16:162-218. 1938.
10. WULFF, H. D., Chromosomenstudien an der Schleswig-Holsteinischen Angiospermen-Flora. III. Ber. Deutsch. Bot. Ges. 57:84-91. 1939.

CURRENT LITERATURE

Experimentelle Cytologie. By HANS H. PFEIFFER. A New Series of Plant Science Books, edited by Frans Verdoorn. Vol. IV. Waltham, Mass. (formerly Leyden): Chronica Botanica Co., 1940. Pp. 243. Figs. 28. \$4.00.

This text constitutes a survey of the scope, methods, and results of contemporary research in experimental plant cytology. The purpose of Dr. PFEIFFER is to fill a gap in plant science literature. He admits, however, that an exhaustive review of experimental work on plant cells with its diverse viewpoints cannot be presented in such a small volume; many results of a purely chemical, physical, and genetical nature are therefore omitted. Even so, a vast amount of literature concerning the application of experiment to problems of cellular and tissue development has been assembled, and many phenomena have been correlated and presented. Selection, arrangement, and theoretical treatment of the material reflect the author's previous methodologic-analytical efforts and are predominantly based on his own research and on that of KÜSTER, FREY-WYSSLINGH, and other European investigators of normal and pathological plant and cell structure.

The first three chapters are introductory, dealing with the scope of experimental cytology: the material, methods, and agents used in the experimental production of cellular reactions or formation of cell structures, and form and size changes of the cell, nucleus, and plastids. Chapters follow on the basic physiological properties of protoplasm and the living cell, such as structure, physico-chemical and biological properties of protoplasm; osmosis; permeability; viscosity; vital staining; electrophysiological experiments; ionic, pH, and radiation effects.

In the remaining portion changes in visible cell structure and typical cellular functions, as well as the submicroscopical processes underlying them, are discussed in relation to different experimental treatments, in chapters on cell growth; differentiation, dedifferentiation and necrosis; explantation of cells; mitosis; cytokinesis; dislocation experiments; micrurgy; extraction and introduction of cellular components and substances.

Emphasis is laid on the general morphological and physiological behavior of the cell rather than on cell division, the nucleus and the chromosomes. The attitude is analytical and comparative throughout, with no preference for any particular experimental method, and—though the treatment is often somewhat sketchy—the danger of oversimplifying complex phenomena has been avoided. Lists of important papers and texts are given, but unfortunately citations for most of the papers mentioned in the text are omitted. Several topics, which the student of present-day cytology might expect to find, are not discussed, such as effects on the cell of natural and synthetic growth substances, enzymes, and colchicine, as well as the work on nucleic-acid metabolism, polyploidy, and tumor production. In addition to the author and subject index, there is a survey of important cytological contributions from 1748 to 1939.

The volume should prove useful in connection with the present standard texts of general and comparative plant cytology, none of which has yet synthesized our somewhat unconnected knowledge in the various fields of experimental cytology. Investigators will find it helpful as an outline of experimental methods and general results, and for its many references; it should prove stimulating to the students of morphology and morphogenesis, and to the general cytologist it may serve as a botanical supplement to JAMES GRAY's textbook.—ROBERT BLOCH.

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NUTRITION OF THE MYXOMYCETES. II. RELATIONS BETWEEN PLASMODIA, BACTERIA, AND SUBSTRATE IN TWO-MEMBERED CULTURE¹

A. L. COHEN

(WITH ONE FIGURE)

Introduction

Under natural conditions, the myxomycetes are constantly found associated with microörganisms of many kinds, including bacteria, yeasts, molds, and protozoa. This association prevails not only during the vegetative stages of the myxomycetes but in the fruit as well, for sporangia have been found to contain the spores of bacteria and fungi and the cysts of protozoa, all extruded from the plasmodium during its fructification (22). Although this continual association with microörganisms has been noticed by many (19), the amount of sound correlative work on such relations is relatively slight. It has been mentioned previously (5) that factors other than the quantity of bacterial food operate in mixed cultures, and the present paper is an attempt to elucidate these factors.

Literature review

The reports of other workers on the relationships between myxomycete plasmodia and bacteria have been reviewed previously (5). It is established that plasmodia and myxamebae can ingest and digest a number of different microörganisms, but relatively few detailed studies have been made on the general types most readily ingested, the conditions governing ingestion, and the other factors concerned.

In considering the relations in two-membered cultures, two statements previ-

¹ Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, no. 188.

ously discussed (5) may be at once dismissed: CHRZĄSZCZ'S (2) claim that the microorganisms and the myxomycetes are in competition for the supplied medium, and HENNEBERG'S (8) conclusion that the myxomycete feeds mainly on the metabolic products of the microorganisms rather than on the microbes themselves.

SKUPIENSKI (26) has maintained that a symbiosis exists between each species of myxomycete and a particular species of bacterium, chiefly because plasmodia were often found associated each with a single species. From reports by other workers, and from the evidence presented here, it would not seem that a constant association in gross culture or in nature is alone a reliable criterion of symbiosis.

A further consideration of the relations between myxomycetes and bacteria apparently shows no correlation between the morphological types of the bacteria and the ability of plasmodia to assimilate them. WATANABE (29) found that although the five most suitable bacteria in his graded list were Gram positive, they agreed in no other respect; others found Gram-negative bacteria to be equally well assimilated (28, 14; cf. 5). WATANABE'S conclusions were based on the chemotaxis of plasmodia in association with the bacteria on nonnutrient agar; the suitability of the bacteria as food was judged by the degree to which the plasmodia followed and fed on the streak microorganisms; but, as shown later, this method cannot be accepted as altogether reliable.

Because of the paucity of data on the myxomycetes it is necessary to turn to studies of other types of organisms for light on the relations in two-membered cultures—the Acrasieae and the protozoa.

A thorough study of the relations between *Dictyostelium discoideum* Raper and the associated bacteria has been made by RAPER (23, 24). Although he found that *D. discoideum* is constantly associated with *Vibrio alkaligenes* Lehm. and Neum., it grew much better when certain other bacteria were used as associates. In two-membered cultures with bacteria the growth of the *Dictyostelium* was limited chiefly by the hydrogen-ion concentration of the substrate. *Dictyostelium* would not feed on those bacteria which raised the pH to a considerable degree; but if the increased alkalinity were prevented by buffering or using fermentable sugars, excellent development often occurred. The physical nature of the bacterial colony was also an important factor, for the *Dictyostelium* would not grow on bacteria which formed large amounts of slime, or which formed tough matted colonies (for example, *Bacillus mycoides*) difficult for the myxamebae to ingest. As with the myxomycetes, the feeding ability could not be correlated with morphological characters such as flagellation, spore formation, and Gram reaction.

The Gram reaction of the bacteria appears to be of little importance in determining the feeding behavior of the protozoa, although there is an impression that Gram-negative bacteria are in general more favorable as food than Gram-positive ones. This belief stems chiefly from the researches of OEHLER (20), who found that

of five amebae studied, one or two definitely preferred Gram-negative forms. In later papers (summarized in 21) OEHLER found the ciliates, *Colpoda steinii* and *C. cucullus*, could be fed on a great variety of bacteria, refusing only the acid-fast ones. Furthermore, it was with the Gram-positive *Bacillus subtilis* that HARGITT and FRAY (7) obtained their best two-membered cultures of *Paramecium*, a finding supported by JOHNSON (13) and LESLIE (17). CLEVELAND (3, 4) also found, in general, better growth of the flagellate *Tritrichomonas fecalis* on Gram-positive bacilli than on Gram-negative bacteria; and as in OEHLER's work, only the acid-fast bacteria were refused (4). To the writer's knowledge, the acid-fast bacteria and the actinomycetes have never been successfully used in the cultivation of protists (21, 4, 25), although LESLIE (17) found a poor growth of *Paramecium* on mycobacteria.

Other factors seem to be more important than the Gram reaction. Slimy bacteria are not easily ingested either by myxomycetes, Acrasieae, or amebae (5, 23, 6). The metabolites of the bacteria are often important; thus highly putrefactive or fermentative bacteria are inimical (7), as well as those which produce certain nitrogenous substances, such as ammonia, urea, and trimethylamine. By using a medium in which these substances are not formed (20), however, it can be shown that the bacteria are not intrinsically unsuitable for food. KIDDER and STUART (15) claim that the pigments of certain chromogenic bacteria are toxic for the ciliate *Colpoda*, and HETHERINGTON (9) has shown that as marked differences can exist between the strains of a particular species as between genera.

It is clear that there are divergent results and opinions on the myxomycetes and other bacterial feeders in their relation to the bacteria. The present paper attempts to clarify these relations. Myxomycete plasmodia possess the great advantage of being macroscopic, and many of the changes taking place in two-membered cultures are therefore easily observed.

Material and methods

Two media were used mainly in these experiments, an oatmeal agar similar to the OA previously described (5) but prepared more exactly, and a yeast-extract-starch agar (YSA). The oatmeal agar was prepared by placing 0.5 gm. of oatmeal and 20 cc. of agar buffered at pH 6.0 (6.0 UA of 5) in each 125 cc. Erlenmeyer flask, which was then plugged and autoclaved. The YSA was prepared by adding to the 6.0 UA 0.5 per cent Difco yeast extract and 2 per cent soluble starch. After the starch and agar had been dissolved by heating, 20 cc. of the medium was placed in each 125-cc. flask and the flasks plugged and autoclaved.

The plasmodia chiefly used were those of *Badhamia foliicola* List., strain 13, and *B. magna* Peck, strain 51. In addition, the following were used in several experiments: *Badhamia* sp., strain 11; *B. utricularis* Berk., strain 22; *Didymium*

squamulosum Fr., strain 14; *Physarum polycephalum* Schw., strain 24; and *Stemonitis axifera* MacBr., strain 21.

In most of the experiments it was desired to use comparatively large inocula of as uniform size as possible; and in view of previous observations of the physiological differences between plasmodia of the same species grown under different conditions, it was further desired that all inoculations of a single species be made from a single stock plasmodium. After some experimentation, it was found that large, pure-culture plasmodia for inoculation could be grown on autoclaved yeast (YA) in large Roux flasks (5). One hundred and fifty cc. of agar buffered to a pH of 6.0 (6.0 UA; 5) was placed in each flask and the flasks plugged and autoclaved. After autoclaving, the flasks were laid horizontally until the agar had solidified. Excessive condensation of water was prevented by drying the flasks in an incubator for several days.

To each flask 1.5 cc. of YA was added under sterile precautions, the yeast suspension distributed over the agar, and each flask inoculated with as large a pure culture inoculum of the particular plasmodium as was feasible. The flasks were incubated horizontally at 25° C. until most of the yeast was consumed and then stored at 12° C. until needed. With experience, large plasmodial fans of about equal thickness throughout could be obtained; and by removing plasmodial fragments of equal area, approximately equal quantities of inoculum were obtained. For this purpose a punch-cutter was employed, made of a short length of nichrome tubing of 5 mm. internal diameter riveted to a brass handle. The cutting edge of the tube was slightly beveled on the exterior side for easier cutting. The punch was flamed, introduced into the flask and cooled, and then as many cuts made as necessary. The material was transferred to the culture vessels by means of a flattened nichrome needle.

After autoclaving to remove the agar, the dry weights of the inocula were obtained by placing the coagulated plasmodia on weighed cover slips and drying to constant weight. The weights thus obtained were between 0.1 and 0.2 mg. in almost all samples.

The bacteria² were suspended either in yeast-extract broth (YE, 5) or in yeast-extract-dextrose (YED, 5) and incubated at least 48 hours at 25° C. before use. They were then inoculated into the flasks of OA by means of Pasteur pipettes. Not more than two or three drops were employed, and the inoculum was dropped directly on the plasmodium and surrounded it. If the plasmodia were not well infected, they would move away from the point of inoculation, leaving the bacteria behind with consequent delay in feeding and growth.

² The bacteria were obtained from a number of sources and are now deposited with the Laboratory of Plant Physiology of Harvard University, under identifying strain numbers.

Results

BACTERIA ASSOCIATED WITH PLASMODIA

In view of statements that several species of bacteria were found in constant association with different plasmodial species of myxomycetes and Acrasieae (22, 23, 26, 28), a qualitative analysis of the microbial flora of gross cultures of plasmodia was undertaken to determine the significance of their association.

TABLE 1

NAME OR ISOLATION NUMBER OF BACTERIA	MYXOMYCETES				
	BADHAMIA SP.	B. POLI- COLA	B. UTRICU- LARIS	PHYSARUM POLYCEPH- ALUM	STEMONITIS AXIFERA
I*.....	+				
II.....	+				
III.....	+				
IV.....	+				
V.....		++	+++	+++	+++
Ps. schuykilliensis.....		+++	+++	+++	+++
Ps. annulata.....		+			
VIII.....					+++
IX.....					+
X.....					+
Ps. ovalis.....			+		+
XII.....					+
XV.....			+		
XVI.....			+		
XVIII.....				++	
B. fulvum.....				+	
XXI.....				+	
XXII.....			+		
B. helvolum.....			+	+	
B. coli.....		+		+	
XXIX.....	+++	+++	+++	+++	

* Characters of unidentified forms.—Gram negative and cephalotrichous: I, II, III, IX, X, XV, XXI, XXII. Peritrichous: V. Gram positive and motility undetermined: IV (?), XVI. Liquefy gelatin: I, II, V, VIII, X, XII, XV, XVI, XXI, XXIII; others do not. Surface growth in yeast-extract agar stab: III, IV, VIII, IX, XXII; others deep growth as well (X, XII, XXIII show only slight deep growth).

The six plasmodia used (with the exception of *Didymium squamulosum*) had all been grown in gross culture on OA for some time. Gross culture sclerotia were placed on OA of pH 6.0 in large moist chambers and kept in diffuse light at 25° C. until most of the plasmodia had fruited, thus demonstrating favorable conditions for completion of the life cycle. Suspensions were prepared from these cultures, plated on the same medium, and incubated in the dark at 25° C. for 5 days. Twenty-five distinct colonies were obtained, which were examined for purity and subsequently cultured and stored on YE or on YED. Table 1 shows the characters of the bacteria and their relative frequency (as indicated by the number of + signs)

with each myxomycete. Duplicate isolates and bacteria of adventitious origin are omitted from the table. In addition to the bacteria listed, there are two species not isolated as just described. No. XXIII was isolated from a culture of *Didymium squamulosum* and *Saccharomyces ellipsoideus* on plain agar. It appeared to be the only bacterial species present. No. XXVIII, *Bacillus pabuli* Schiebl., a starch-hydrolyzing bacillus, was found as a contaminant in oatmeal cultures when the oatmeal was insufficiently autoclaved.

Table 1 indicates that these bacteria are almost all Gram negative, the majority liquefy gelatin, and most of them are motile. Their growth shows nothing very characteristic, and they are—superficially at least—referable to those two great heterogeneous groups of common soil and water bacteria, "*Achromobacter*" and "*Flavobacterium*." It should be noted further that, with the possible exception of the associate of *D. squamulosum*, no one of these bacteria was constantly and exclusively associated with a particular myxomycete; those which were strikingly abundant on one isolation plate were also abundant on several. *D. squamulosum* was not grown in the same manner as were the other organisms, and the conditions in a *S. ellipsoideus* enrichment plate (5) may well have allowed the development of a single bacterium to the exclusion of others.

Preliminary experiments showed that all but a very few of these bacteria formed good two-membered cultures with myxomycete plasmodia. In view of the fact that there were no constant associates, the attempt to study these associations was abandoned. Instead a study was made of the range of known bacterial forms on which the myxomycetes could feed.

STUDIES WITH KNOWN ORGANISMS

As pointed out in the literature review, most general rules determining the ability of protists to feed on bacteria according to "natural" groupings have sufficient exceptions to invalidate them or else insufficient data to support them. It was desirable, therefore, to determine whether any similar generalities could be made for the myxomycetes, and for this purpose a division of the bacteria into reasonably consistent natural groups was necessary. The manuals and systems of bacteriology extant are either out-of-date or else form groups which are hardly consistent (1). These classifications have been discussed by KLUYVER and VAN NIEL (16), who proposed an improved, simplified system. A number of known strains of bacteria were collected to represent as far as possible the tribes and genera defined by these writers. These bacteria and several of the determined species isolated from myxomycete gross cultures were used for further study.

The bacteria and the two myxomycete plasmodia, *Badhamia foliicola* strain 13 and *B. magna* strain 51, were inoculated, each in duplicate, into OA flasks in the

manner described under methods. The results of this experiment are summarized in table 2 for *B. magna*.

Because they grew little if at all on the OA, and furnished no nutrient for the plasmodia which likewise did not grow, the following bacteria used in these experiments are excluded from table 2: *Micrococcus* (*Staphylococcus*) *aureus* Migula, *M. (Staph.) citreus* Migula, *Sarcina lutea* Schroeter, *Sporosarcina* (*Sarcina*) *ureae* Beij., *Betacoccus arabinosaceus* Orla-Jensen, *Bacterium* (*Cellulamonas*) *liquata* McBeth and Scales, and *Zymobacillus macerans* Schardinger.

I. NATURAL GROUPS OF BACTERIA IN RELATION TO FEEDING.—Table 2 shows that there is no general correlation between the tribes and genera of these bacteria and the ability of the plasmodia to feed. There is but one group characteristic which limited the growth of the plasmodia, and that is the very limited growth on OA of several of the cocci: the Micrococcaceae (*M. citreus*, *M. aureus*), the Sporosarcineae (*S. ureae*), the Sarcineae (*S. lutea*), and the Streptococceae (*Betacoccus arabinosaceus*). *Micrococcus albus* and *Micrococcus* sp., which grew fairly well on this medium, also supported fair to good growth of the plasmodia.

The acid-fast bacteria, *Mycobacterium* spp., *Proactinomyces* sp., and *Actinomyces* sp., supported fair to excellent growth of the plasmodia, although these forms have been found unsuitable for the cultivation of other protists (21, 25, 4). The Gram-positive Bacilleae (fig. 1A), with the exception of *B. subtilis* and the Gram-positive *Kurthia zopfii*, supported as good growth as members of the Gram-negative genus *Bacterium*.

II. PHYSICAL NATURE OF BACTERIAL COLONY IN RELATION TO FEEDING.—The physical nature of the bacterial colony often determines the ability of the plasmodia to feed. Thus the slimy capsules formed by several bacteria markedly inhibit feeding (table 2). The young colonies of some bacteria are rather slimy or moist (for example, *Ps. annulata*) but later become more or less dry, with correspondingly increased ease of attack by the plasmodia. The feeding on dry compact colonies differs characteristically from that on moist slimy ones. The plasmodium attacks dry colonies by crawling over them as a fan, and the colony may often be seen through the transparent layer of myxomycete protoplasm. This the writer has called central feeding (table 2), since the plasmodium may cover the colony to the very center. On the other hand, in the peripheral feeding on slimy colonies, the plasmodium banks up against the edge of the colony without penetrating or covering it. The plasmodium is more or less immobilized, and growth is exhibited as a gradual extension of the protoplasm around the edge of the colony from the initial feeding point.

Growth is generally much slower on slimy colonies than on relatively dry ones; but if the plasmodium establishes itself, it may finally creep over and destroy the colony entirely. In such a case the fan is irregular, the strands of plasmodium be-

TABLE 2

BACTERIA (TRIBE, SPECIES, STRAIN NUMBER)	GROWTH OF PLASMODIA	TAXIS TOWARD COLONY	LENGTH OF LIFE (DAYS)	TYPE OF FEEDING	FINAL PH OF CULTURE	CONDITION OF PLASMODIA AT END OF CULTURE
Pseudomonadineae						
<i>Pseudomonas annulata</i> Ches. Ps2.....	+	+	6	p†	4.5	Dead
	+++	+	40	p	7.6	Dead
<i>Ps. schuykilliensis</i> Ches. Ps3.....	+++	+	27	c‡	Sclerotized and dead
	+++	+	27	c	8.0	Sclerotized and dead
<i>Rhizobium trifolii</i> Dang. Rh1 *.....	+	-	6	p	4.9	Dead
	+	+	6	p	5.2	Dead
Micrococcaceae						
<i>Micrococcus albus</i> Buch. Mcc3.....	++	o+	54	c	7.6	Dead, dissociated
	++	+	40	c	7.1	Dead
<i>Micrococcus</i> sp. Mcc1..	+++	+ -	40	c	6.1	Dead
	+++	+ -	40	c	7.6	Dead, dissociated
Mycobacteriaceae						
<i>Mycobacterium</i> sp. Mcr	+	-o	15	c	5.3	Dead
	+++	-o+	40	c	5.6	Sclerotized, disso- ciated
<i>Mycobacterium</i> sp. Mc2	+++	o+	54	c	6.5	Living
	+++	+	54	c	6.5	Living
Bacteriaceae						
<i>Kurthia zopfii</i> Trev. K1.....	+++	+ - +	40	c	3.8	Dead
	+++	+	54	c	5.9	Living
<i>Bacterium acidilactici</i> Mg. Bt6.....	+++	+	54	c	4.2	Dead
	+++	+	40	c	7.4	Dead
<i>B. ceramicola</i> Lundestad Bt3.....	++	+	40	c	6.0	Dead, dissociated
	++	+	40	c	3.9	Dead, dissociated
<i>B. coli</i> (form) Esch. Bt2	+++	o+	40	c	8.3	Dead, dissociated
	+++	+	40	c	8.3	Dead, dissociated
<i>B. prodigiosum</i> DeT Trev. Bt4.....	++	o+	40	p	6.3	Sclerotized
	6	7.4	Dead
Bacilleae						
<i>Bacillus cereus</i> Frank. Frank. Bc3.....	++	+	9	c	4.8	Dead
	+	+	54	c	3.9	Living, dissociated
<i>Bac. danicus</i> Löhn. West. Bc1.....	+	+	54	c	4.0	Living, dissociated
	+++	+	27	c	5.2	Living
<i>Bac. megatherium</i> De Bary Bc5.....	+++	+	40	6.1	Living
	++	+	54	3.9	Living, dissociated
<i>Bac. mycoides</i> Flüggé Bc4.....	++	+	40	c	3.9	Living, dissociated
	++	+	40	c	3.9	Living, dissociated

* Slimy colony.

† Peripheral feeding.

‡ Central feeding.

TABLE 2—Continued

BACTERIA (TRIBE, SPECIES, STRAIN NUMBER)	GROWTH OF PLASMODIA	TAXIS TOWARD COLONY	LENGTH OF LIFE (DAYS)	TYPE OF FEEDING	FINAL PH OF CULTURE	CONDITION OF PLASMODIA AT END OF CULTURE
<i>Bacillaceae—Continued</i>						
<i>Bac. pabuli</i> Schiebl. Bc2	+	—	15	6.7	Dead
	++	o—	27	7.4	Dead
<i>Bac. subtilis</i> Praz. Bc6..	+—	15	o	Dead
	+—	15	o	Dead
<i>Aerobac. polymyxa</i> Praz.
Abc1*.....	++	+	54	p	Dead
	+++	+	40	p	6.8	Dead
<i>Actinomycetales</i>						
<i>Proactinomyces</i> sp.
PrAc1.....	—	6	5.0	Dead
	++	+	6	c	4.6	Dead
<i>Actinomyces</i> sp. Ac1...	+++	o+	27	c	7.5	Dead
	+	+	13	c	7.9	Dead
<i>Undetermined</i>						
<i>Capsulated coccus</i> X2*	+++	o+	40	p	7.9	Dead
	+++	o	40	c	6.8	Dead
<i>Capsulated bacterium</i>
Bbtr1*.....	+	+	40	p	7.0	Dead
	+	+	40	p	6.9	Dead

ing twisted and lax (fig. 1B), having much the same appearance as a plasmodium on too moist a substrate. Contrasted with this is the "normal" appearance of a plasmodium (the fan flat), smoothly rounded at the front edge, and gradually breaking up into long, regular main strands converging at the rear.

It was thought at first that the dry, tough, nonwetting colonies of the actinomycetes and the dry, tough, adherent colonies of *Bacillus mycoides* would not be attacked, or at least only with difficulty. On the contrary, *B. mycoides* in association with the plasmodia furnished an excellent example of central feeding, and the actinomycete colonies were also completely eaten (fig. 1D II).

III. TACTIC BEHAVIOR OF PLASMODIA AND FEEDING.—Tactic behavior toward bacterial streaks was used by WATANABE (29) as a criterion of the ability of plasmodia to feed on bacteria. The plasmodia, placed on nonnutrient agar, were encircled with a streak of the bacterium to be tested. The suitability of the bacterial food was determined by noting whether or not the plasmodium followed the streak and fed or crossed it without feeding. In this connection, note the tactic behavior of the plasmodia toward the bacteria listed in table 2. Here a positive chemotaxis is indicated by +, a negative taxis by —, and no definite taxis by o.

A plasmodium often moved away from the point of inoculation, then by the third day returned to it (after the bacteria had begun to grow), left again, and again returned. Often there seemed to be no very definite taxis, but the plasmodi-

um wandered around until it came in contact with the colony and began feeding. Sometimes a plasmodium temporarily ceased feeding. In many instances feeding began only because the colony had spread to such an extent that the plasmodium could not very well move about without coming in contact with it.

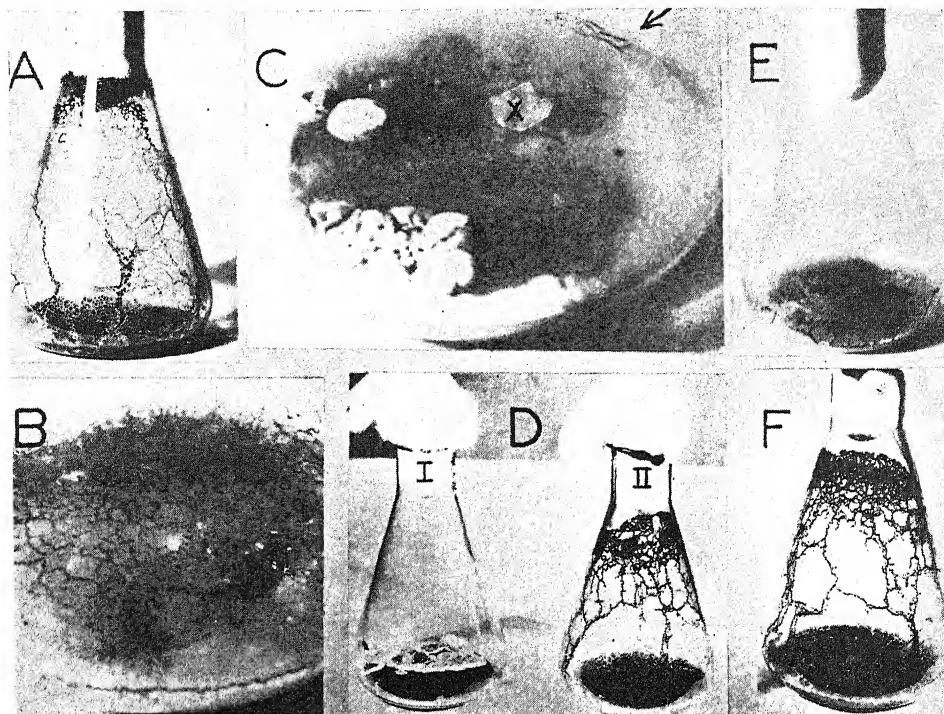


FIG. 1.—A, *Badhamia foliicola* on Gram-positive *Bacillus danicus*, oatmeal agar, 27 days' growth; plasmodium sclerotizing at top of flask. B, same on unidentified slime-forming bacterium (Bbt1), oatmeal agar, 20 days' growth. C, *Badhamia magna* and *Actinomyces* sp. on yeast-extract-starch agar: *Actinomyces* colonies in foreground; plasmodium inoculated at X; arrow points to starving plasmodium; 4 weeks after inoculation. D, *B. foliicola* and *Actinomyces* sp., oatmeal agar, 46 days after inoculation: I, plasmodium not in contact with colonies, starved; II, plasmodium cleared *Actinomyces* from agar, now sclerotizing. E, dissociation of *B. foliicola* in two-membered culture with *Mycobacterium* sp. (Mc1); 27 days' culture, dissociant beginning growth. F, same as E; 41 days' culture.

Although WATANABE (29) found that not one of seventeen different myxomycetes would ingest *Bacillus megatherium* on plain agar, the writer has shown that when they are forced to live in unavoidable contact with it, *Badhamia foliicola* and *B. magna* will feed on this bacterium.

A similar and even more striking example of the independence of taxis and feeding is found in the association of *Physarum polycephalum* with two species of yeast, *Saccharomyces ellipsoideus* Hansen and *Torula aclotiana* Kufferath. Although the

plasmodium was successfully established in two-membered culture with these yeasts, it would still feed on neither when streaked on plain agar plates. There are, of course, exceptions to the independence of chemotaxis and feeding. Most of the plasmodia in culture show pronounced positive chemotaxis to *S. ellipsoideus* streaked on plain agar; this fact was used to advantage in the enrichment technique (5).

When a plasmodium was transferred to a fresh substrate, it almost invariably and immediately left the old portion transferred with it for the new. In two-membered cultures on OA the plasmodium generally stopped crawling to erode the first grains with which it came in contact. This movement was slowed down so much that the bacteria with which the plasmodium had been inoculated grew faster than the plasmodium could rid itself of them by migration.

The first cultures were made on YSA because of its advantages over OA. Being nonparticulate, the YSA could be rendered wholly liquid by autoclaving, the coagulated plasmodium removed, and its dry weight obtained as a quantitative expression of growth. Furthermore, the plasmodium hydrolyzed the starch of this medium to simple sugars utilizable by the bacteria. Since the degree of hydrolysis was dependent on the quantity of plasmodium, the richness of the medium was controlled to some extent by plasmodial growth, and the danger of the plasmodium being swamped by heavy growth of the bacteria was lessened. But the several advantages of YSA were outweighed by one very serious disadvantage: the plasmodium crawled away from the inoculum so speedily that it freed itself of the bacteria in the process. In the absence of a well-defined positive taxis toward the colony growing at the site of inoculation, the plasmodium starved with only a few centimeters separating it from its bacterial food (fig. 1C). These cultures on YSA were made with *Badhamia magna* and all the bacteria of table 2.

Eleven of these cultures were selected at random and the myxomycete plasmodia from them inoculated into YED broth tubes. All except one remained sterile, confirming the statement that the myxomycetes had freed themselves of the bacteria by crawling away from the point of inoculation. The possibility remained that the immediate growth of many of the bacteria had produced toxic substances in sufficiently large concentration to inhibit feeding. Another group of twenty-five cultures was selected which included most of the bacteria of table 2, and the myxomycete reinoculated adjacent to each colony. The colony at the same time was spread around the myxomycete so that there would be no chance of the plasmodium avoiding constant contact with the bacteria. The myxomycete grew in eighteen of these twenty-five flasks, and on the same bacteria as in the OA flasks (fig. 1D); therefore toxicity of the bacteria could not have been a factor.

The factors influencing growth in two-membered cultures were sufficiently numerous as soon as a plasmodium, a substrate, and a bacterium were brought to-

gether, without adding the complication of the myxomycete freeing itself of its associate, as in most of the cultures on YSA. For this reason OA was adopted as a substrate, as on it the plasmodia, on leaving the site of inoculation, generally stopped creeping and started to erode those oatmeal grains with which they first came into contact.

IV. CHEMICAL SUBSTANCES ELABORATED BY BACTERIA.—Evidence was obtained that metabolites produced by the bacteria may be inhibitory for growth. *Bacterium acidi-lactici* and *Badhamia foliicola* were inoculated into flasks containing media of the following compositions:

A. yeast extract.....	0.25%
dextrose.....	2.0%; original pH 5.4
agar.....	2.0%; final pH 4.1
B. yeast extract.....	1.0%
dextrose.....	2.0%; original pH 5.7
agar.....	2.0%; final pH 5.0
C. yeast extract.....	1.0%
dextrose.....	0.5%; original pH 5.8
agar.....	2.0%; final pH 7.9

At the end of 3 weeks there was excellent growth in the flasks containing medium A, but none in the flasks of media B and C. Because of its high final pH, no just comparison may be made between medium C and the other two. However, the final pH of medium A or B is within the range in which *Badhamia magna* grows well, and growth was inhibited solely by quadrupling the concentration of yeast extract. This concentration is not itself toxic to *B. foliicola*, and therefore lack of growth and death must have been due to metabolites elaborated by *Bacterium acidi-lactici* from the nitrogenous yeast extract.

Bacterium prodigiosum allowed a fair growth of both *Badhamia foliicola* and *B. magna*, although it is the same strain that KIDDER and STUART found extremely toxic for *Colpoda* and *Daphnia* (15). Nor did the plasmodia take up the colored granules sufficiently to change their natural color, in contrast to the observations of KAMBLY (14) on the plasmodia of several myxomycetes, and of RAPER (23) on the myxamebae of *Dictyostelium discoideum*.

V. DISSOCIATION OF PLASMODIA.—Another phenomenon in culture somewhat parallels the adaptations mentioned elsewhere (5). Because the phenomenon resembles to a great degree the dissociation of bacterial species in which a colony of bacteria gives rise to a variant colony differing in certain respects from the parent, the same name is applied.

In several of the cultures the plasmodia started growth and then fragmented into several smaller plasmodia, one or more of which grew abundantly, while the others did not feed and finally died or were lost in the greatly increased mass of the

"dissociants." Plasmodia have also been observed to grow fairly well in two-membered cultures and then apparently to die, while much of the bacterial mass remained unconsumed. Close inspection revealed that there were minute living plasmodial fragments left, and these dissociants began growth anew in the same culture and on the same bacterial mass (fig. 1E). The final growth sometimes surpassed the original, the entire mass being consumed (fig. 1F) and the oatmeal grains completely hollowed. The vegetative state ended in most cases by the plasmodium sclerotizing on the walls of the flask. These dissociations have been noted in *Badhamia foliicola* feeding on *Micrococcus albus*, *Bacillus megatherium*, and *Mycobacterium* sp. (Mcr); and in *B. magna* on *Micrococcus albus*, *Micrococcus* sp., *Mycobacterium* sp. (Mcr), *Bacterium ceramicola*, *B. coli*, *Bacillus cereus*, *Bac. danicus*, *Bac. megatherium*, and *Bac. mycoides*.

INTERRELATIONS BETWEEN BACTERIA AND PLASMODIA

Under this heading are placed those phenomena in culture not readily assignable to the bacteria or to the plasmodia but to an interaction between both.

I. HYDROGEN-ION CONCENTRATION OF MEDIUM.—It was stated earlier (5) that the myxomycete plasmodia did not grow on those bacteria which raised the pH to an unfavorable degree (above 7.4). Yet in these later studies the final pH of two-membered cultures in which there was excellent development of the plasmodium was often extremely high (8.3 for *Badhamia magna* on *B. coli*). Furthermore, duplicate cultures may in certain cases have widely different final hydrogen-ion concentrations (table 2). Often within the same culture the pH varied from place to place, being generally comparatively high near the bacterial colony, low near a portion of a living plasmodium not in contact with the colony, and high near any mass of dead plasmodium. The single values given in table 2 were all taken near the plasmodia, preferably living ones if they were alive when the cultures were discontinued.

Since the quinhydrone electrode was used for these determinations, it is possible that the potentials were the combined expressions of hydrogen-ion activity and oxidation-reduction activity, were it not that determinations made with a glass electrode and with acid-base indicators were in thorough agreement with the quinhydrone determinations.

An analysis of the changes of pH produced in the substrate by the plasmodium alone was necessary, and examples are given in table 3. These support the conclusion that in presence of carbohydrates, the living, active, plasmodium lowers the pH. After death, presumably through the autolysis of its nitrogenous components, it raises the pH.

Thus, it might be expected that since both the plasmodium and the bacterium affect the pH of the medium, the relative sizes of the two inoculations would in-

fluence the final pH of a culture. Experiments with *Badhamia foliicola* and *Pseudomonas schuylkilliensis* confirm this supposition. *Ps. schuylkilliensis* inoculated into flasks of OA raised the pH of the medium to 7.4 in about 3 days. Small (0.5×1 mm.) inoculations of *B. foliicola* into these cultures died immediately. In the next experiment, larger inoculations of the *Badhamia* were made into the flasks and the quantity of added *Pseudomonas* suspension reduced. The plasmodia began growth, but were soon outstripped by the *Pseudomonas* and died in a week. In the final experiment, given in table 2, the plasmodia were inoculated in relatively large quantity compared with the *Pseudomonas*, and their feeding limited the accumula-

TABLE 3
CHANGE IN PH OF OATMEAL AGAR AFTER GROWTH OF
PLASMODIA; ORIGINAL PH 5.9-6.1

CULTURE	FINAL PH	REMARKS
<i>Badhamia foliicola</i> (pure) on oatmeal agar. . .	5.4	Small (starving) plasmodium
<i>B. foliicola</i> on oatmeal agar+autoclaved yeast	5.8	Plasmodium recently dead; pH taken adjacent plasmodium
Same culture.	5.5	pH taken at distance from plasmodium
<i>B. foliicola</i> on oatmeal agar+autoclaved yeast	4.8	Plasmodium alive; pH taken adjacent plasmodium
Same culture.	5.6	pH taken at distance from plasmodium
<i>B. magna</i> on oatmeal agar, two-membered culture with <i>Sacch. ellipsoideus</i>	6.9	Good growth; plasmodium dead, disintegrated
<i>S. ellipsoideus</i> alone on oatmeal agar.	4.8	Thirty days' growth

tion of two great numbers of bacteria, with the result that the plasmodia grew until the nutrients of the culture were exhausted.

As in previous experiments (5), it was found that plasmodia inoculated immediately from a medium of pH 6.0 into media of pH 7.4 or higher promptly died. If the plasmodia were transferred successively through media, each of a slightly more alkaline reaction than the preceding, they could live and grow at a final pH of 7.4. The same adaptation probably operates in two-membered cultures in which plasmodia have grown with alkali-forming organisms. The cultures listed in table 2 were kept until the plasmodia had died, sclerotized, or fruited in most of them (some as long as 2 months). During this time, in which a balance existed between the myxomycetes and the bacteria, adaptations to the slowly changing conditions could easily have occurred.

II. CARBOHYDRATE CHANGES IN MEDIUM.—It was found previously (5) that myxomycete plasmodia in a pure state on OA could erode the grains, but without growing appreciably, and it was concluded that bacteria or other microorganisms

were necessary for complete utilization of the medium. The more extensive erosion occurring in two-membered cultures offered two possibilities: (a) the myxomycete could utilize the oatmeal as an accessory nutrient provided bacteria or yeasts were present; or (b) the microorganisms themselves could act on the medium in such a manner that it could then be utilized by the myxomycete.

It was found that the yeasts and the majority of the bacteria cultivated on OA could not erode the grains exposed at the surface. An exception was the bacterium isolated from oatmeal and identified as *Bacillus pabuli*. Yet the plasmodia grew equally well, and in many cases better, in two-membered culture with microorganisms which alone had no effect on the grains. That the bacteria did not contribute something to the medium which would allow growth in pure culture, and that they did not prepare the starchy grains so as to make them more easily assimilated, was demonstrated by the following experiments.

Bacterium coli was grown in pure culture on OA and the cultures divided into three lots: one lot was autoclaved and shaken to distribute the bacteria throughout the medium; another lot was heated to 60° C. to kill the bacteria; a third lot was heated to 60° C. and the bacteria carefully washed off the surface with sterile water. *Badhamia foliicola* was inoculated into all three series of flasks. In those lots in which the bacteria were autoclaved and suspended throughout the medium, and in those where they had been killed by heating to 60° and washed off, there was no growth and little erosion of the starchy contents of the grains. In the third lot, in which the bacteria remained on the surface and were easily accessible, the plasmodia grew, devoured the heat-killed bacteria, and eroded the surface grains extensively.

An experiment with autoclaved yeast confirmed this result. The yeast was added to flasks of OA, 0.5 cc. per flask, and the flasks agitated to distribute the suspension over the surface. Equal-sized inocula of *Badhamia foliicola* were placed in each flask and in control flasks of OA alone. The flasks were incubated until the yeast had been completely consumed. In the control flasks, in which the plasmodia were minute and starving, the average number of grains eroded was seven; in the flasks containing YA, in which there was a fair growth of the plasmodia, the average number eroded was twenty.

It seemed that the disappearance of the starch as indicated by erosion of the grains was proportional to the size of the plasmodium, and this was confirmed by inoculating pure culture plasmodia ranging in size from minute inocula to those covering an area of several square centimeters into OA flasks. The number of eroded and hollowed grains varied roughly with the size of the plasmodia.

The digestion of cooked starch by plasmodia had been observed by LISTER (18) and was confirmed by the experiments just described. But in addition, the writer noticed that some microorganisms, particularly yeasts, when grown on a starchy

medium such as oatmeal or potato agar were benefited by the presence of a plasmodium.

It was evident that not only did the yeast support growth of the plasmodium, but the plasmodium also supported growth of the yeast. Neither plasmodia killed by heating and placed in flasks of OA, nor extracts of plasmodia, produced better growth of the yeast than did the OA alone. Therefore the myxomycetes were changing the medium to a more nutritious one for the yeast. These observations were confirmed by pouring suspensions of living yeast cells (*S. ellipsoideus*) in properly buffered YSA into petri dishes. No growth of the yeast occurred, for the essential sugar was absent. On introduction of any one of the plasmodia obtained in pure culture (5, table 1) there was growth of yeast in the track of the plasmodium and concomitant disappearance of the starch, as shown by the iodine test.³

Within a two-membered culture an ecological balance is maintained between both members, provided care is taken that neither member is introduced in so large quantity that it overwhelms the other. This balance is shown by an opposing effect of the plasmodium on the raising of the pH by bacteria, and in a cycle wherein the plasmodium begins to feed on the microörganism, at the same time breaking down starch into simple sugars, which promote growth of the microörganism so that it is further used by the plasmodium, the cycle continuing until the substrate is staled or exhausted.

Discussion

It has been shown that in undisturbed gross cultures of myxomycete plasmodia there are several common bacteria which dominate the flora. However, in contrast to the statement of SKUPIENSKI (26) that each plasmodium has its characteristic bacterium, a bacterium common in one culture is also common in several. A seeming exception—the association of bacterium XXIII with *Didymium squamulosum*—has been discussed earlier.

The gross cultures of plasmodia other than *D. squamulosum* were grown on OA, which may be considered also as a specialized medium because of its high carbohydrate and low protein content. The medium was buffered at pH 6.0; the temperature was approximately 25° C. Thus those bacteria which require rather restricted conditions for growth (that is, higher temperature), a medium rich in proteins, and greater alkalinity, were excluded in favor of the organisms which could grow better under these conditions. These bacteria, as might be expected, were the common Gram-negative pseudomonads and non-spore-forming peritrichs typical of water and moist plant remains. It seems apparent, therefore, that the cultures were enrichment cultures for these bacteria as well as for the plasmodia, and that the single criterion of a more or less constant association is not sufficient to

³ Data confirming the elaboration of a diastase by plasmodia will be given elsewhere.

postulate a symbiosis. If there were any closer relationship than this, it would be expected that the plasmodia could not grow at all well with organisms other than their natural associates. Indeed the opposite is true, for two-membered cultures could be made with astonishingly diverse microorganisms, including the yeasts, acid-fast bacteria, and actinomycetes. These facts are supported by the demonstration of HOWARD and CURRIE (11, 12), who showed that *Physarum polycephalum* can feed on a number of fungi other than those with which it is naturally found.

A much better case could be made for symbiosis in the two-membered cultures. First, it has been shown that under certain conditions the shift of the hydrogen-ion activity is kept from going too fast in one direction or the other; and second, the extracellular digestion of the starch by the myxomycete results in the enrichment of the medium for those second members which can utilize sugar, the increased growth of the second member being then reflected in an increased growth of the myxomycete.

Nevertheless the writer does not consider a two-membered culture to be a symbiosis. It seldom happens that the plasmodium sclerotizes or fruits with much of the bacterial mass uneaten. If the bacteria are not cleared, the plasmodium generally dies. But if the plasmodium does grow abundantly, it is at the final expense of the bacterium whose colonies are completely cleared from the medium. Whether or not the myxomycete enriches the medium for the bacterium depends on the bacterium and the medium. If sugar is supplied for an organism that can utilize sugar, there is no need for the starch-destroying power of the myxomycete. If the microbe can easily dispense with sugar, as is the case with many pseudomonads, the starch hydrolysis is of no avail. Furthermore, the association is neither close nor constant and may be made with a plasmodium and any one of a number of very different organisms.

The complexity of the food relations and the difficulty of study are further enhanced by the adaptations mentioned previously (5) and by the dissociations found here.

The writer is not the first to report the breaking up of a plasmodium, for LISTER (18) recorded similar behavior in the case of *Badhamia utricularis* fed on the unfavorable *Agaricus rubescens* and *A. fascicularis*. The plasmodium in both cases fed to some extent, then broke up into portions, some of which died and others began feeding again.

HOWARD (10) also has noted *Physarum polycephalum* plasmodium breaking up into portions, some of which continued to feed and others to sclerotize or fruit. The writer has observed the same behavior with this and other plasmodia, and in addition has seen *P. polycephalum* in two-membered culture break up into two portions, the strands of which were interwoven without fusing. One portion later

sclerotized while the other continued to feed. SMART (27) has observed a closely related phenomenon in the case of *Fuligo septica*. The plasmodium of this species under certain conditions goes through several color changes, ranging from white to rusty yellow. White plasmodia will fuse with other white plasmodia and yellow ones will fuse with yellow, but cross fusions do not take place, although the plasmodia may be of the same clone.

Summary

1. In a study of myxomycete gross cultures, twenty-five different bacteria have been isolated, nearly all Gram negative, and most of them motile and possessing the property of liquefying gelatin. In the gross cultures on oatmeal agar there was found to be no specific association of a particular microorganism with a particular plasmodium, and those bacteria common in association with one plasmodium were common in association with several. These bacteria possessed the characters of the common soil and water bacteria, and it is concluded that there was no symbiosis between a bacterium and a plasmodium, but that the medium used was favorable to both.

2. A further study made with thirty-one different bacteria belonging to diverse groups showed no feeding preference of the plasmodia on the bases of morphological and natural groups. Unlike other known bacterial feeders, the plasmodia were found to grow on the mycobacteria and actinomycetes. The positive or negative chemotaxis toward the colony provided no indication as to whether the myxomycete can feed on the particular bacterium.

3. The final pH of the medium was controlled by both the plasmodium and the bacterium. If the pH of the medium were changed too speedily, the myxomycete could not grow. But by adjusting the medium and the relative sizes of the plasmodial and bacterial inoculations, the pH changed rather slowly and a balance could be obtained in which the plasmodium broke down the starches of the medium into sugars utilizable by the associated microorganisms, which grew more abundantly and in turn furnished more food for the plasmodium.

4. Only two factors were found which consistently limited growth of the plasmodium. The myxomycetes grew poorly on bacteria which formed slimy colonies and on bacteria which grew poorly on the media employed.

5. A phenomenon comparable with bacterial dissociations was found in some cultures, namely, the plasmodium grew to some extent, most of it then died, but small living fragments began growth anew.

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LITERATURE CITED

1. BERGEY, D., Bergey's manual of determinative bacteriology. Baltimore. 1934.
2. CHRZĄSZCZ, T., *Physarum leucophaeum ferox*, eine hefefressende Amöbe. Centralbl. Bakt. Abt. II. 8:431-441. 1902.
3. CLEVELAND, L. R., The separation of a *Tritrichomonas* of man from bacteria; its failure to grow in media free of living bacteria; measurement of its growth and division rate in pure cultures of various bacteria. Amer. Jour. Hyg. 8:256-278. 1928.
4. ———, The suitability of various bacteria, molds, yeasts, and spirochaetes as food for the flagellate *Tritrichomonas fecalis* of man as brought out by the measurement of its fission rate, population density, and longevity in pure cultures of these microorganisms. Amer. Jour. Hyg. 8:990-1013. 1928.
5. COHEN, A. L., Nutrition of the myxomycetes. I. Pure culture and two-membered culture of myxomycete plasmodia. Bot. Gaz. 101:243-275. 1939.
6. CUTLER, C., and CRUMP, L., The qualitative and quantitative effects of food on the growth of a soil amoeba (*Hartmanella hyalina*). Brit. Jour. Exp. Biol. 5:155-165. 1927.
7. HARGITT, G., and FRAY, W., The growth of *Paramecium* in pure cultures of bacteria. Jour. Exp. Zool. 22:421-454. 1917.
8. HENNEBERG, W., Hefe fressende Amöben eines Schleimpilzes (*Physarum leucophaeum* Fr.) und hefe fressende Thieramöben. Wochenschr. Brauerei. 18:159-161; 173-175. 1901.
9. HETHERINGTON, A., The rôle of bacteria in the growth of *Colpidium colpoda*. Physiol. Zool. 7:618-641. 1934.
10. HOWARD, F. L., The life history of *Physarum polycephalum*. Amer. Jour. Bot. 18:116-133. 1931.
11. HOWARD, F. L., and CURRIE, M. E., Parasitism of myxomycete plasmodia on the sporophores of Hymenomycetes. Jour. Arnold Arb. 13:270-284. 1932.
12. HOWARD, F. L., and CURRIE, M. E., Parasitism of myxomycete plasmodia on fungous mycelia. Jour. Arnold Arb. 13:438-447. 1932.
13. JOHNSON, W., Studies on the nutrition and reproduction of *Paramecium*. Physiol. Zool. 9:1-14. 1936.
14. KAMBLY, P., The color of myxomycete plasmodia. Amer. Jour. Bot. 26:386-390. 1939.
15. KIDDER, G., and STUART, C., Growth studies on ciliates. I. The role of bacteria in the growth and reproduction of *Colpoda*. Physiol. Zool. 12:329-340. 1939.
16. KLUYVER, A., and VAN NIEL, C. B., Prospects for a natural system of classification of bacteria. Centralbl. Bakt. Abt. II. 94:369-403. 1936.
17. LESLIE, L. D., Nutritional studies of *Paramecium multimicronucleata*. II. Bacterial foods. Physiol. Zool. 13:430-438. 1940.
18. LISTER, A., Notes on the plasmodium of *Badhamia utricularis* and *Brefeldia maxima*. Ann. Bot. 2:1-24. 1888.
19. MACBRIDE, T., and MARTIN, G., The myxomycetes. New York. 1934.

20. OEHLER, R., Amöbenzucht auf reinem Boden. Arch. Protistenk. 37:175-190. 1916.
21. OEHLER, R., Gereinigte Zucht von freilebenden Amöben, Flagellaten und Ciliaten. Arch. Protistenk. 49:287-296. 1924.
22. PINOV, E., Rôle des bacteries dans le développement des certains Myxomycètes. Ann. Inst. Pasteur 21:622-656; 686-700. 1907.
23. RAPER, K. B., Growth and development of *Dictyostelium discoideum* with different bacterial associates. Jour. Agr. Res. 55:289-316. 1937.
24. ———. Influence of culture conditions upon the growth and development of *Dictyostelium discoideum*. Jour. Agr. Res. 58:157-198. 1939.
25. SEVERTZOVA, L., The food requirements of soil amoebae. Centralbl. Bakt. Abt. II. 73: 162-179. 1928.
26. SKUPIENSKI, F. X., Recherches sur le cycle évolutif des certains myxomycètes. Paris. 1920.
27. SMART, R., The influence of external factors on the behavior and development of the myxomycetes. Thesis, Harvard. 1935.
28. VOUK, V., Die lebensgemeinschaften der Bakterien mit einigen höheren und niederen Pflanzen. Naturwiss. 4:81-87. 1913.
29. WATANABE, A., Über die Bedeutung der Nährbakterien für die Entwicklung der Myxomyceten-Plasmodien. Bot. Mag. Tokyo 46:247-255. 1932.

DISTRIBUTION OF CALCIUM OXALATE CRYSTALS IN *RICINUS COMMUNIS* IN RELATION TO TISSUE DIFFERENTIATION AND PRESENCE OF OTHER ERGASTIC SUBSTANCES

FLORA MURRAY SCOTT

(WITH FORTY-TWO FIGURES)

Introduction

Ensheathed crystals of calcium oxalate in *Ricinus* were first described by ROSANOFF (17) in 1865. In the original figures the druses appear inclosed within distinct envelopes, supported approximately in the center of the cell by short stalks attached to the wall. In 1888, WAKKER (23) adopted the tonoplast method of DE VRIES and laboriously studied cells plasmolyzed to the point of protoplasmic rigidity by prolonged treatment with KNO_3 and eosin. He states that crystals always arise in vacuoles, and that stalks and sheaths are present only in cells devoid of living protoplasm and are therefore dead cell artifacts. WAKKER concludes: "Es wäre immerhin besser diese Balken als Rosanoff'sche Balken zu unterscheiden, und so werden wir die denn weiter auch nennen."

During the next 10 years discussion centered around the origin of crystals, whether in the cytoplasm or in vacuoles, and the nature of the crystal sheath, whether a dead cell artifact or a normal growth. WITTLIN (24) reviews this literature briefly and, in a survey of crystal formation in medicinal plants, outlines the development of crystals in *Ricinus*. The extensive literature of more recent years has been surveyed by MEYER (8) and NETOLITSKY (10), and the crystallography of calcium oxalate has been discussed in detail by FREY (5).

The mechanism of the origin of oxalic acid and calcium oxalate remains a problem for the phytochemist and the physiologist rather than for the anatomist. Plant anatomy, however, is concerned with the distribution of calcium oxalate throughout the living plant, since a study of distribution in space and time serves to throw light on the relation of crystal deposition to other dominant phases of tissue metabolism (3). As NETOLITSKY (10) points out, developmental studies in individual plants are curiously lacking.

The present paper is concerned with the development of calcium oxalate crystals and their distribution in the seedling and mature plant of *Ricinus* in relation to tissue differentiation and the presence of other dominant ergastic substances. Etiolated shoots and seedlings are also examined.

Material and methods

Ricinus grows freely throughout the entire year in southern California, and seedlings and mature plants are available at all seasons. Sections of fresh material from different regions—stem, root, leaves, inflorescence and fruiting axis, fruit and seed, seedling axis, and cotyledons—were examined under ordinary light, polarized light, and with dark-field illumination. Etiolated shoots and seedlings were also examined. Fresh material treated with 1 per cent osmic acid may be mounted in Berlese medium.¹ Fatty substances appear intensely blackened, calcium oxalate crystals stand out clearly, and the wall contraction inevitable in xylol-Canada-balsam preparations is avoided. In the detailed examination of the younger tissues, imbedded material is necessary, of course. Navashin's solution or osmic acetic solution² proved satisfactory.

Sections of varying thickness were examined unstained or after treatment with haematoxylin or with safranin and fast green. Ergastic substances were identified microscopically and by microchemical tests. They include (in addition to calcium oxalate) fatty substances, starch, tannin, sugar, the pigments chlorophyll and anthocyanin, and the wall materials cellulose, lignin, suberin, and cutin.

Observations

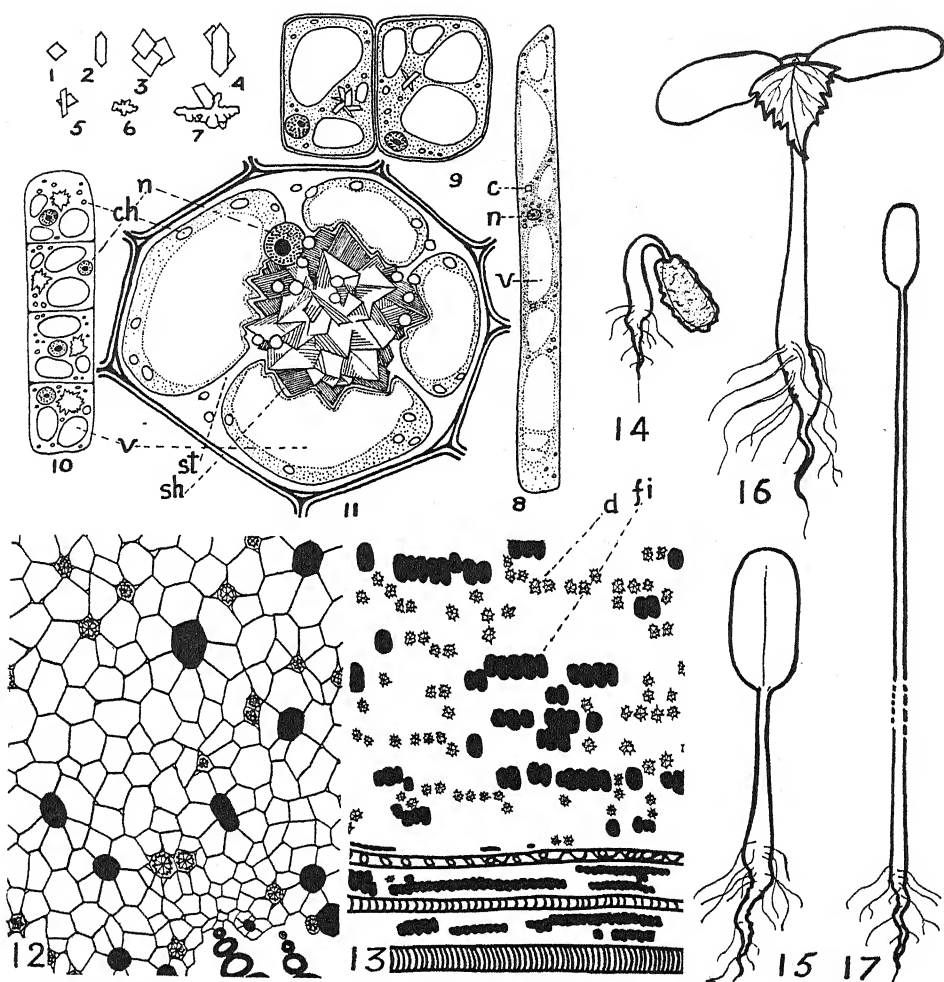
TYPES OF CRYSTALS AND THEIR DEVELOPMENT

Two types of crystals are present in *Ricinus*, druses and solitary crystals. These conform to the crystallographic types described by FREY (5). The former are distributed throughout parenchyma tissues in general but are particularly conspicuous at the nodes, while the latter are practically restricted to the cells adjacent to the fibers of the secondary phloem.

DRUSES (figs. 1-11).—In the apical meristem of the shoot the first traces of crystal formation appear as minute points of light, visible only under the polarizing microscope. The earliest identifiable druses occur in the vacuolating cells of the third internode and in the leaf and bud sheath primordia of the apical region. Druse development is most readily observed in the leaf; in tangential sections of the projecting leaf veins, all stages of development may be found in one or a few sections. The druse originates as a single crystal, which may be cubical, bipyramidal, rodlike, or needle-like in form, and $2-3\ \mu$ in greatest diameter. Whatever the outline, the crystals in undamaged cells are suspended in protoplasmic strands in vacuolating cells. In this connection the penetration of neutral red is useful in defining the cell sap and vacuoles, and thus delimiting the protoplasmic strands.

¹ H₂O (distilled), 100 cc.; gum arabic, 60 gm.; chloral hydrate (saturated solution), 100 cc.; glycerin, 40 cc.

² Osmic acid (1%), 1 part; acetic acid (2%), 1 part.



FIGS. 1-17*.—Figs. 1-11, stages in druse development: figs. 1, 2, individual crystals; figs. 3-5, crystal aggregates; figs. 6, 7, dendritic growth; figs. 8-10, developing crystals within cell (8, two single crystals in protoplasmic strands; 9, crystal aggregates; 10, miniature druses from longisections of leaf veins); fig. 11, druse in mature parenchyma cell at petiole base. Figs. 12, 13, distribution of fat and calcium oxalate in pith of primary tissues, eleventh and twelfth internodes: fig. 12 (transection) and fig. 13 (longisection) show alternation in spaces of druses (stars) and fat idioblasts (solid black), circles, and ellipses. Figs. 14-16, stages in development of green seedling: fig. 14, hypocotylary arch stage, cotyledons inclosed in endosperm; fig. 15, straight hypocotyl, cotyledons green; fig. 16, plumule developing. Fig. 17, etiolated seedling; actual length 32 cm.

* Abbreviations: *c*, cambium; *ch*, chloroplast; *cr*, crystal; *d*, druse; *f*, fiber; *fi*, fat idioblast; *n*, nucleus; *s*, starch; *sh*, sheath; *sp*, sieve plate; *st*, stalk; *v*, vacuole. All drawings with camera lucida.

If the protoplasmic strand is broken, the crystal may be released into the vacuole and Brownian movement is observed. The number of crystals in a cell is variable. As a general rule, in isodiametric cells one crystal only is present, somewhere near the center of the cell. In elongated cells two to four crystals may occur, distributed at approximately equal intervals throughout the protoplast.

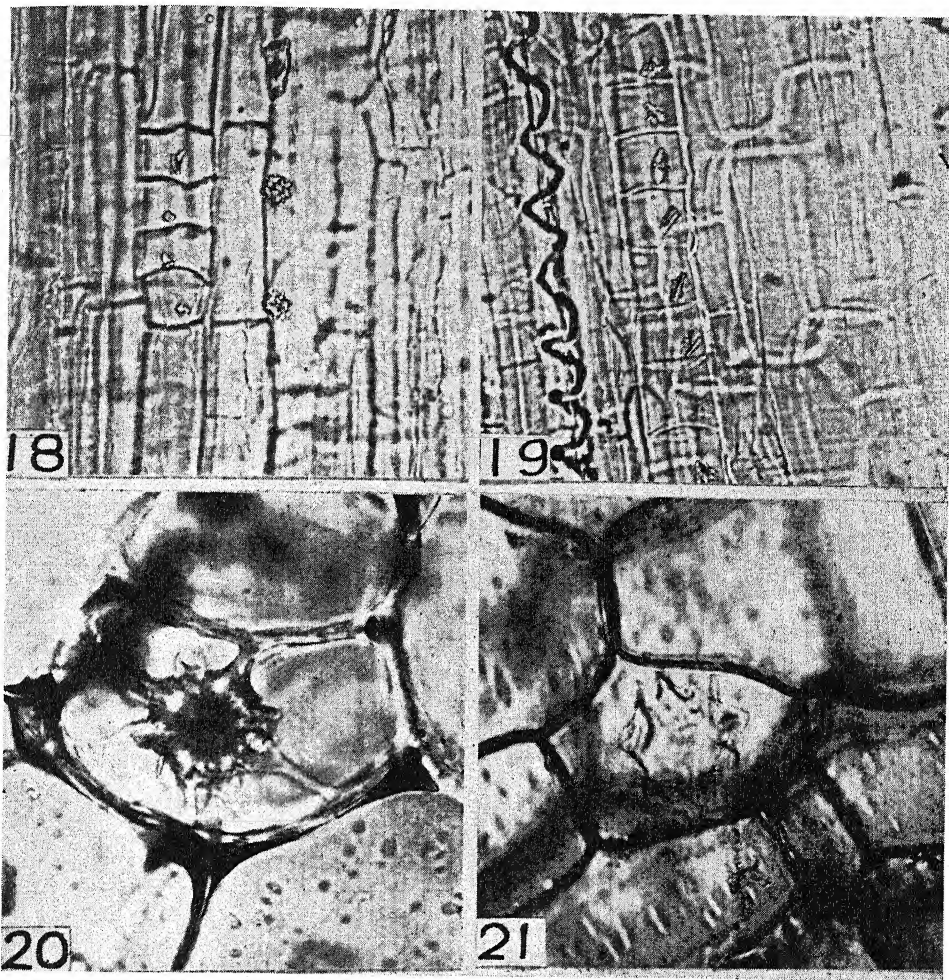
The further development of the druse is variable. An aggregate may appear, consisting of two or more similar or dissimilar individual crystals. On the other hand, dendritic growth appears to be equally common, and increase in volume takes place by haphazard accretions. This dendritic phase is soon masked by more regular crystal growth, and the miniature druses—a few microns in diameter—are exact replicas of the fully developed rosette crystal aggregates (figs. 18, 19).

The larger druses are inclosed within a cellulose sheath and anchored to the cell wall by one or more hollow cellulose stalks. It is impossible by current microchemical and microscopic methods to determine exactly at what stage cellulose deposition first begins. Small crystals about 3μ in diameter already possess a tenuous sheath, demonstrable after careful solution of the crystal but not identifiable microchemically. Since crystal idioblasts and the inclosed druses increase in volume during tissue differentiation, the crystal sheaths and the stalks must be plastic in nature. Pectic substances and possibly some protein are therefore probably present in the original sheath and are masked by the later development of cellulose. The hollow stalk is presumably laid down around a protoplasmic strand. The strand itself is either retracted from the mature stalk or becomes indurated and persistent within it (figs. 20, 21). That cessation and resumption of crystal growth may take place is indicated by the occasional appearance of pitted cellulose sheaths. Crystal points may project beyond a heavy meshlike sheath, and suggest that localized breakdown of the original envelope has occurred, followed by renewed crystal growth.

When some of the larger druses are treated with IKI and H_2SO_4 , followed by H_2SO_4/CP , dissolution of the sheath is not complete. A brownish envelope remains which stains slightly with Sudan III and may therefore indicate sheath suberization. This suberization is erratic in occurrence and is not limited to certain regions nor to certain sizes of crystals. The wall of the idioblast itself remains unchanged, and shows—so far as observed—no trace of suberin or of lignin, as noted by WITTLIN (24) and others.

Young crystal idioblasts differ microscopically from ordinary parenchyma cells only in size, as will appear later, and in their crystal content. Nucleus, chloroplasts, mitochondria, and vacuoles are normal in number, appearance, and distribution in the protoplasts. As the crystal develops the nucleus may remain central in position, adjacent to the druse, or may retreat to the wall, generally adjacent to a stalk base.

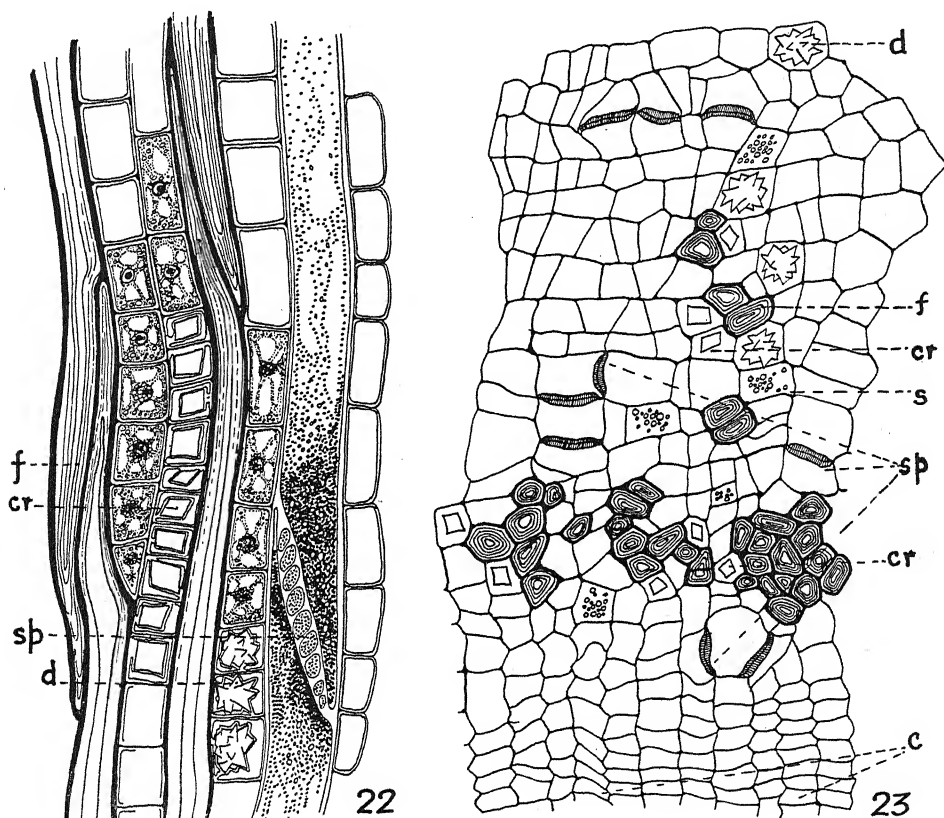
SOLITARY CRYSTALS (figs. 22, 23).—Solitary crystals are abundant only in the secondary phloem adjacent to the phloem fibers. They appear in vertical files of isodiametric parenchyma cells, which differentiate from cambial fusiform initials.



FIGS. 18-21.—Developing and mature crystals: figs. 18, 19, druse development, individual crystals, crystal aggregates, dendritic growth, and miniature rosettes from longisection of leaf vein; fig. 20, druse from mature parenchyma cell showing stalk, sheath, and air spaces at cell corners (dark shadows); fig. 21, sheath left after solution of crystal in similar cell.

The number of cells in any vertical file ranges from three or four to twelve or more, and in general the total length of the file may equal the length of the adjacent fiber. The walls of the septate elements thicken and may lignify unilaterally, either partially or completely.

Like druses, solitary crystals appear first as simple units, cubical or rhombohedral, $1-3\ \mu$ in size when first detected microscopically. There is no evidence that solitary crystals must necessarily arise by recrystallization of druses (12), although an occasional minute druse may occur in this tissue. In position the solitary crystals are similar to druse crystals, central in isodiametric cells, evenly

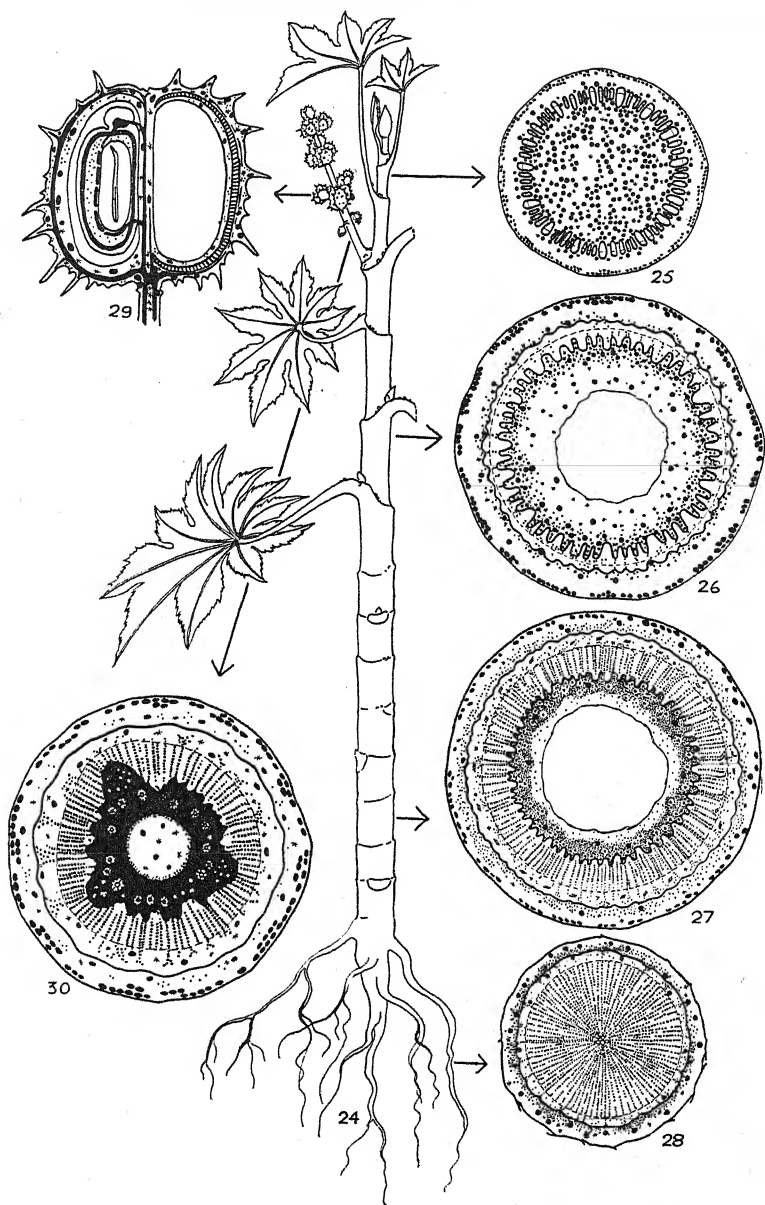


FIGS. 22, 23.—Solitary crystals in secondary phloem: fig. 22, longisection; fig. 23, transection

spaced in elongated cells. Crystal sheaths of undetermined composition remain after solution of these small crystals. As in the case of the druse, the mature solitary crystal is inclosed within a heavy cellulose sheath, practically fused to the cell wall or visibly attached by one or more very short stalks. The exact time at which sheath formation begins remains so far undetermined.

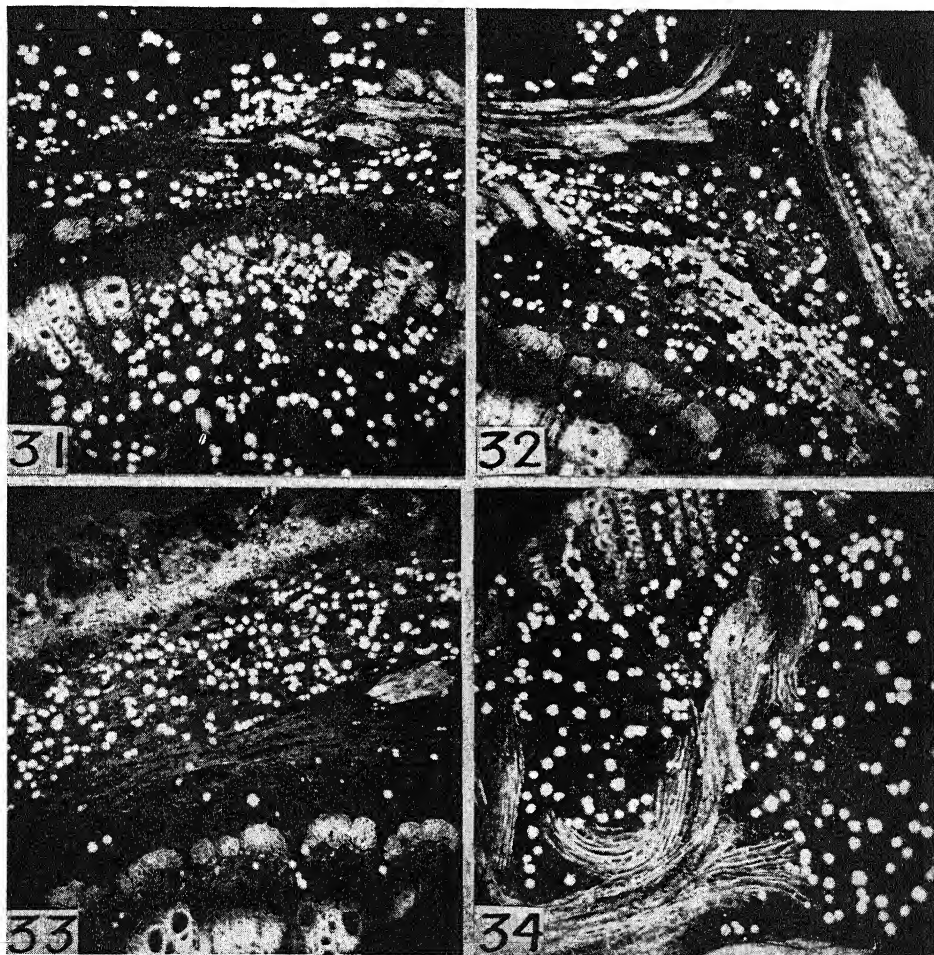
CRYSTAL DISTRIBUTION IN MATURE PLANT

Since crystal distribution is considered in relation to tissue differentiation, it is necessary to review briefly the anatomy of the plant as a whole (figs. 24-30). The



FIGS. 24-30.—Distribution in mature plant of calcium oxalate (crosses), fatty substances (larger black circles and ellipses), and starch (stippling or solid black in pith of fig. 30): fig. 24, young fruiting plant, eight internodes within terminal bud; fig. 25, transection, eleventh internode, druses and fat idioblasts alternating spatially in pith; fig. 26, transection, fifteenth internode, starch beginning to appear in outer pith and rays; fig. 27, transection, leafless stem, starch completely filling rays, and in outer pith partially masking fat idioblasts and crystals; fig. 28, transection, root; starch is dominant reserve material throughout entire root system; fig. 29, longisection, green fruit; calcium oxalate, fat, and starch relatively sparse; fig. 30, transection, fruiting branch as seed ripening begins; pith and rays completely packed with starch (solid black and stippled, respectively). All drawings diagrammatic.

main shoot of a typical vigorous plant of one season's growth may reach a height of 1-2 m. or more and a diameter of 4-6 cm. at the junction of shoot and root at ground level.



FIGS. 31-34.—Distribution of druses and fat idioblasts in region of node (blackened with osmic acid; semi-dark field): fig. 31, transection below leaf gap; fig. 32, leaf base and leaf traces; fig. 33, cortical region; fig. 34, part of vascular nodal septum.

Although the phyllotaxy is $2/5$, only rarely $3/8$, the first three regions of the stem each include eight internodes, 1-8, 9-16, and 17-24 (15). The leaves reach their maximum size about the level of the twenty-fourth node, and leaf fall may begin at the twenty-fifth. Lenticel and cork formation are evident at varying distances below this level. Flowering may begin when the plant is about 1 m.

high, and the fruits develop rapidly. The tap root is well developed and bears numerous secondary roots. Tissue differentiation is considered in the following sections in relation to crystal distribution.

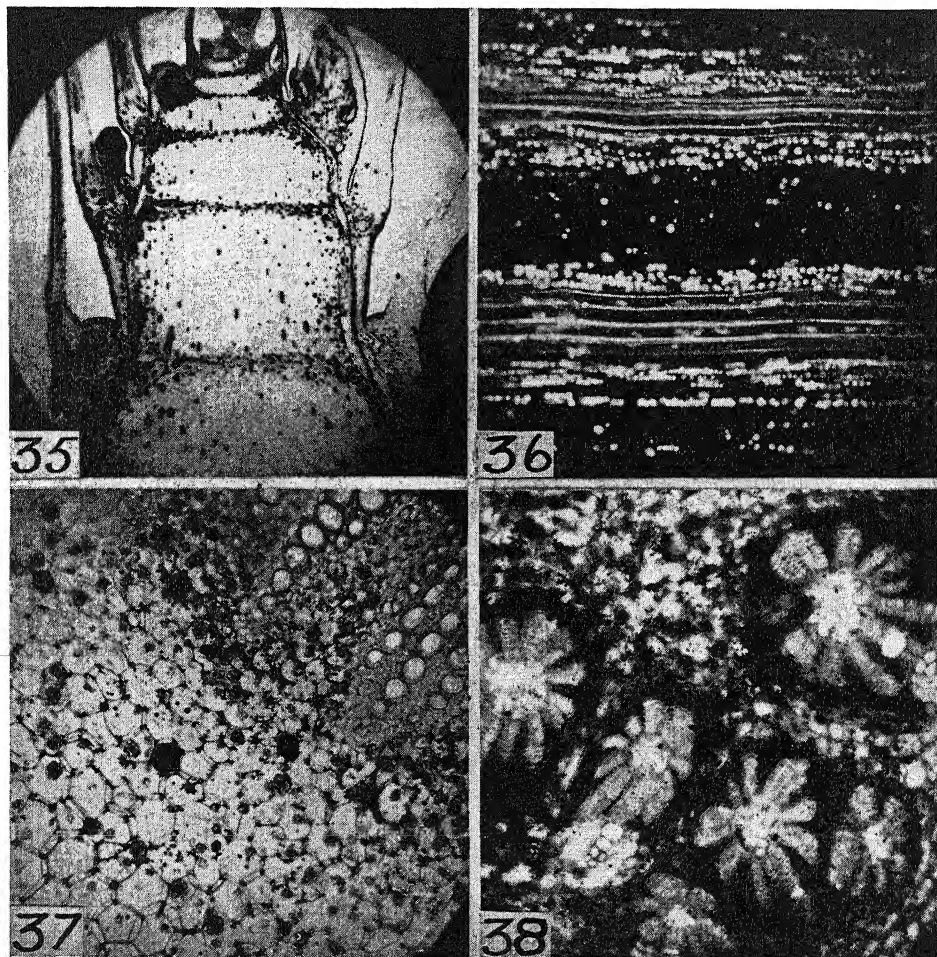
APICAL BUD (fig. 35).—Within the bud sheath, cell division is active in meristematic and in vacuolating dividing cells (14). Vascular differentiation is evident in the spiral elements at the base of the third left primordium and thence downward (18). Phloem keeps pace with xylem development. Anthocyanin, chlorophyll, tannin, sugar, and protein (Millon's reagent) are present in this region in small amounts, but the outstanding ergastic material is unsaturated fat. Fat idioblasts immediately reduce osmic acid. They occur sporadically but in gradually increasing amounts in the epidermis, pith, and other differentiating parenchyma cells and are particularly abundant at the level of the nodes. In pith and in parenchyma in general they may occur either as isolated cells or as vertical files two to ten or more cells in height, and one or occasionally two or more cells in width. Careful control of the osmic reaction shows the fatty substances in an extremely fine state of dispersion throughout the entire cytoplasm. This fine dispersion, of course, is the cause of the complete blackening of the cell contents.

The first traces of calcium oxalate in the apical region are presumably minute points of light visible in the differentiating parenchyma tissues under dark-field illumination or with crossed nicols. At the level of the third node (the region of the earliest vascular differentiation), minute druses $1-3\ \mu$ in diameter may be identified in the pith. In the succeeding internodes crystal idioblasts appear first as isolated cells which alternate in space with the fat idioblasts. They increase in number and, in the seventh and older internodes, vertical crystal files of varying length and width roughly parallel the fat-containing elements (figs. 12, 13).

In the pith, both fat and crystal idioblasts are usually surrounded by a ring of normal parenchyma cells. Those surrounding the fat cells contain some finely dispersed fatty substances and darken slightly with osmic acid. No such reaction occurs around the druse-containing cells. The two types of idioblasts therefore appear as metabolic centers of calcium oxalate and fat formation, respectively, and between them partition the territory of the pith parenchyma.

PRIMARY STEM (figs. 25, 39, 41).—In the primary stem the following tissues are clearly defined: epidermis, hypodermis with occasional large tannin sacs, cortical chlorenchyma and collenchyma, starch sheath, pericyclic parenchyma and fibers, vascular tissues, rays, and pith. The pith, except at the nodes, is hollow from the tenth internode onward. The persistent nodal septa consist of a complex network of vascular tissue in a ground tissue of living parenchyma. Differentiation of the vascular tissue of the node keeps pace with that of the stem (16). Chloroplasts are large and numerous in cortical chlorenchyma and in certain cells of the phloem

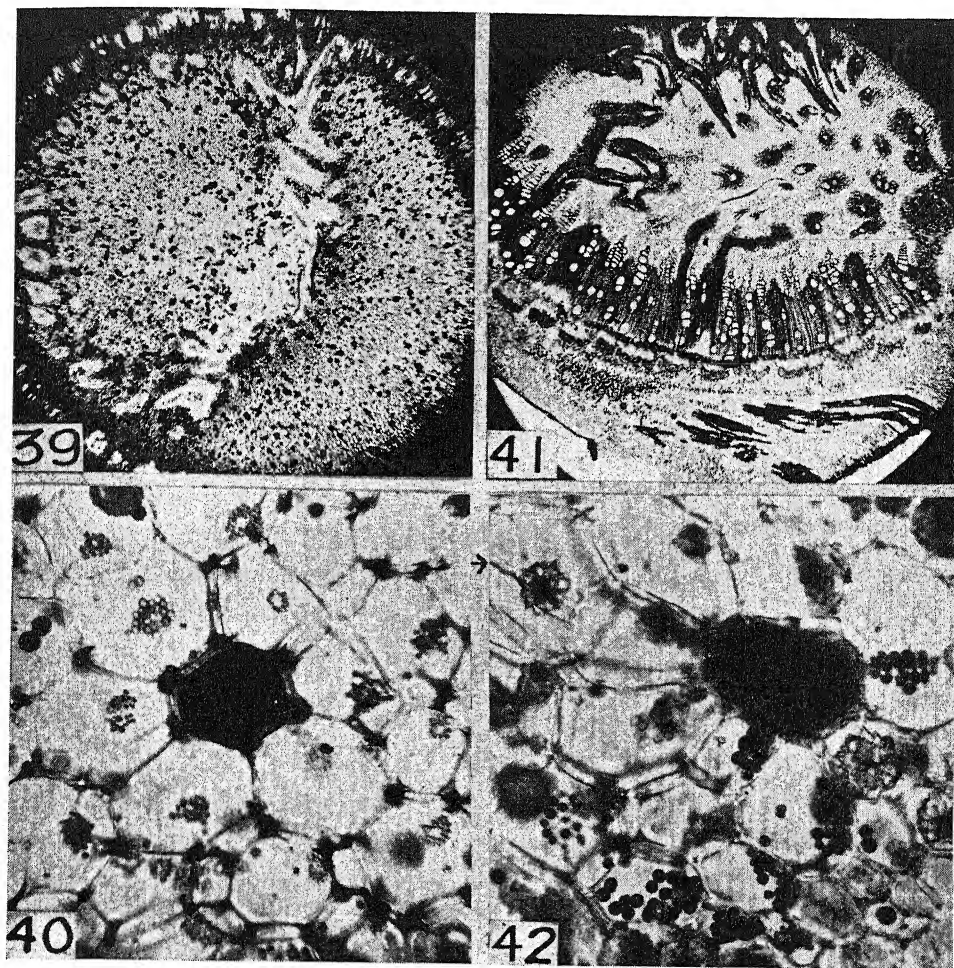
rays. A few smaller chloroplasts appear in the collenchyma, in the pith, and in the remaining parenchyma of the vascular system.



FIGS. 35-38.—Sections of stem apex, leaf vein, secondary region of stem, and inflorescence axis: fig. 35, longisection of stem apex showing fat idioblast distribution; faint grey dots are calcium oxalate druses; fig. 36, longisection of leaf vein showing druses in adjacent parenchyma (dark field); fig. 37, transection of seventeenth internode; starch grains beginning to appear in rays and outer pith; fig. 38, transection of fruiting axis; parenchyma cells adjacent to medullary bundles packed with starch.

The pattern of distribution of ergastic substances as outlined in the region of the apical bud is now clearly defined. Fat and calcium oxalate are the dominant metabolic products. In addition the following other substances are present in lesser amounts: (a) Transitory starch grains, minute in size, appear in the photo-

synthetic tissues. (b) Anthocyanin occurs sporadically in epidermal and subepidermal cells. (c) Tannin is present in the same tissues and is also found more or



FIGS. 39-42.—Fig. 39, eleventh node showing vascular network, fat idioblasts, druses faintly grey; fig. 41, older node, vascular network after treatment with phloroglucin and HCl. Figs. 40, 42, sections of pith; fat idioblast (blackened by osmic acid) surrounded by starch-containing cells; note stalked druse in fig. 42.

less sparingly in the pith; specialized tannin sacs of considerable size occur either immediately beneath the epidermis or deeper within the cortical chlorenchyma. (d) Fehling's solution tests indicate the general distribution of sugar, with an apparent maximum in the region of leaf base and nodal septum.

Fat idioblasts occur in the epidermis and throughout the parenchyma tissues and are very abundant at the level of the nodes and the leaf bases. Druses appear in parenchyma and also reach their greatest concentration in the nodal region. In the pith the spatial alternation of calcium oxalate druses and fat idioblasts is generally maintained (figs. 12, 13). In this region, therefore, it appears that the maximum distribution of both fat and calcium oxalate occurs at the level of leaf base and node and coincides with the expected and apparent maximum of sugar (figs. 29-32).

REGION OF SECONDARY THICKENING (figs. 26, 27, 31-34).—The spiral vessels of the primary system are succeeded by pitted vessels and fibers. Secondary phloem consists of alternate bands of functional sieve tubes with adjacent companion cells and parenchyma, and of phloem fibers with adjacent crystal idioblasts (figs. 22, 23). As in *Nicotiana* (4), secondary sieve tubes possess steeply oblique sieve plates. "Sieve-tube" starch is invariably present. Phloem fibers arise from fusiform elements, thicken basally as to walls by the addition of successive lamellae, and appear to maintain throughout their development a plastic growing tip as in *Boehmeria* (1). Adjacent to the fibers lie the solitary crystal idioblasts (3) and near them phloem parenchyma cells with large chloroplasts.

As regards ergastic substances, calcium oxalate and fat still remain dominant and reach their maximum density at the level of leaf base and node, but in starch distribution a marked change is apparent. Hitherto starch has appeared only as minute transitory granules within the chloroplasts. At this level measurable grains of storage starch begin to fill up the cells of the outer pith and xylem rays and are also evident to some extent in the cortical chlorenchyma (figs. 37, 40, 42).

LEAFLESS MATURE STEM (fig. 27).—Secondary thickening continues normally in the vascular cylinder, and on the surface of the stem lenticels become conspicuous. A cork cambium, epidermal or subepidermal in origin, appears and sooner or later becomes continuous.

In the ergastic substances of this region a striking change is at once apparent. Starch, hitherto a minor product, is now dominant. The rays and practically all the parenchyma tissues are completely filled with starch grains. Calcium oxalate druses and fat cells are now masked more or less by the surrounding starch-filled cells. Here and there empty crystal sheaths indicate the disappearance of druses, and starch grains develop within the former crystal idioblast. This disappearance of crystals, observed somewhat infrequently in the stem, is very common—as will appear later—in the inflorescence and fruiting axis.

Druse cells, fat idioblasts, and normal pith parenchyma cells do not grow at the same rate. In the youngest internodes the scattered fat idioblasts increase in volume more rapidly than in the surrounding parenchyma cells (table 1). They expand against the latter and maintain contact with them, so that no intercellu-

lar spaces develop at their surface. Later, beyond the bud sheath, the parenchyma cells make up for lost time, grow rapidly, equal and eventually surpass the fat cells in volume. Intercellular spaces now develop between parenchyma cells and idioblasts. In contrast to this, the druse idioblasts of the fourth internode are equal in size to the pith parenchyma cells, but they do not keep pace with the growth of the latter. Intercellular spaces consequently are apparent practically from the time that the druse idioblasts first appear.

TABLE 1
COMPARISON OF MAXIMUM SIZE OF NORMAL PARENCHYMA
AND DRUSE AND FAT IDIOBLASTS IN PITH
OF VARIOUS INTERNODES

NO. OF INTERNODE	"AVERAGE" DIAMETER OF CELL (IN MICRONS)*		
	NORMAL PARENCHYMA	FAT IDIOBLAST	DRUSE IDIOBLAST
1 (apex).....	8	8
4.....	21	27	21
8.....	38	50	34
10.....	57	52	38
12.....	99	70	43
14.....	133	85	66
18.....	228	152	76
28.....	228	114*	57

* Cells of pith may be isodiametric or radially elongated. In the latter case, "average" diameter is taken as the mean of the two dimensions of the cell apparent in longitudinal section. Typical largest cells in internodes were measured.

All cells, fat idioblasts, druse cells, and normal pith parenchyma continue to increase in size until about the level of the fifteenth internode. The rate of growth of fat cells thus exceeds that of pith parenchyma in the first eight internodes within the bud sheath. The growth of druse idioblasts, in contrast to this, lags behind that of the parenchyma cell from the time of the first appearance. Shrinkage of fat idioblasts appears occasionally in the oldest tissues (table 1).

LEAF (fig. 36).—In the leaf veins calcium oxalate crystals are evident in practically all stages of development. In the caducous stipular sheath of the terminal bud, druses occur adjacent to the sheath traces. They appear at the leaf base of the third and succeeding leaves. As soon as intercellular spaces appear generally throughout the lamina, druses appear sparsely in the mesophyll itself and abundantly in the parenchyma which surrounds the vascular bundles of the projecting veins. The center of the leaf consists of a network of converging veins, similar in its complexity to the nodal vascular septum. As in the latter, calcium oxalate is again abundant.

Throughout the petiole tissues the distribution of druses is similar to that in the stem, the greatest number occurring in the central pith and occasional crystals elsewhere in the parenchyma. The number increases toward the petiole base.

Just as in the stem, fatty substances in the petiole and leaf base parallel the crystal distribution. They appear intermittently in epidermis and subepidermis, in cortex and vascular parenchyma. In the pith they alternate in space with crystal idioblasts and increase markedly in abundance toward the leaf base.

Within the lamina the maximum distribution of fat idioblasts occurs in the region of maximum concentration of calcium oxalate, namely, in the network of convergent veins at the leaf center and along the projecting veins themselves. In the latter, fatty substances appear in the pith of the vascular cylinder, in xylem and phloem parenchyma, and above and below the vascular strands. Fat is also present in certain epidermal and mesophyll elements.

In the leaf bases of older leaves, starch accumulation begins and keeps pace with the deposition in the older internodes. The same masking of crystals by starch grains may occur sooner or later, prior to leaf fall.

INFLORESCENCE AND FRUITING AXIS (figs. 30, 38).—The inflorescence axis bears staminate and pistillate flowers protected in the bud by scale leaves only. It resembles the vegetative shoot in essential structure but differs in certain characters which are obvious during the later fruiting stages: (1) Since it lacks vegetative leaves the axis is devoid of the complex vascular network of the vegetative nodes. (2) The inflorescence axis does not increase rapidly in diameter as does the main shoot. As a result the pith remains a solid continuous cylinder, usually with a central core of nonliving air-filled cells. Lignification of walls is common throughout the entire tissue. (3) The vascular supply of the flower pedicels appears as a group of medullary bundles traversing the outer pith. The bundles are concentric in type; xylem vessels and a heavy sheath of fibers surround a central strand of phloem. In the latter, sieve tubes are at once conspicuous by their starch granule content.

The change from fat to starch metabolism, evident during the secondary thickening of the vegetative stem, is paralleled in the growth of the fruiting branch. The axis differs in ergastic materials during various stages of growth.

In the early stages of development of the unopened buds and inflorescence axes the ergastic substances are similar in distribution and amount to those of the young vegetative shoot. Thus fatty substances are evident in epidermis, subepidermis, and pith, and also sparsely distributed in cortical and vascular parenchyma. Calcium oxalate is notable in the pith, and occasional crystals may appear in other parenchyma cells. Conspicuous starch grains delimit the starch sheath. Smaller granules of phloem starch are present in the sieve tubes, while minute granules may also be detected in the cortical chlorenchyma.

Active accumulation of starch begins with the setting of fruit. (The green fruit measures approximately 2×2 cm., including the spines.) Here, in addition to an increasing number of endodermal starch grains and sieve-tube granules, conspicuous grains are evident throughout the outer pith, particularly around the medullary bundles. Similar grains are also present in the xylem rays. The air-filled central cells, lignified and dead, naturally remain devoid of starch, but the fat and calcium contents of some cells still remain *in situ*. In the chlorenchyma the starch grains are now abundant. By the time the fruit is ripe the outer pith and the ray cells are solidly packed with starch grains. Sieve-tube starch is still evident, and many cortical phloem parenchyma cells are also heavily loaded. During this period the majority of calcium oxalate druses in the pith disappear and the crystal idioblasts become filled with starch grains (2). To begin with, two or three starch grains appear within the druse cell. The crystals now begin to break down and the starch grains increase in number. Occasionally empty crystal sheaths may be detected, surrounded by a "corona" of starch grains. Increasing starch accumulation naturally ends by obscuring all traces of the original crystal sheath.

In the developing fruit itself and in the seed, calcium oxalate, fat, and starch appear more or less abundantly at different stages of growth. The wall of the spiny, 3-locular dehiscent fruit consists of photosynthetic and vascular tissues and a solid layer of sclerenchyma fibers which eventually harden the capsule wall (fig. 29). In developing fruits (3–5 mm. or more in length) both calcium oxalate and fatty substances are rather abundant. Their concentration, as in other parts of the plant, is at a maximum in the region of vascular tissue, the carpel traces and the vascular network of the fruit base. Starch may appear in older fruits but is relatively sparse.

In the seed, in contrast to the fruit, calcium oxalate is rarely observed at any stage of development (6), and fatty substances similar to those of the vegetative tissues are likewise sparse. In the young ovule the inner integument is filled with starch. This disappears as the integuments begin to harden into the testa, and as—at the same time—protein and fat accumulate in the endosperm around the developing embryo. Endosperm fat differs from vegetative fat since it does not immediately reduce osmic acid. Occasional druses, very small in size, may occur near the micropyle, and minute crystals—detectable only under dark-field or polarized light—appear in the adherent nucellus. No traces of such crystals are observed at any time in the endosperm.

Root (fig. 28).—The young root is generally tetrach in structure (20), and the tissues, except for collenchyma and chlorenchyma, are essentially similar to those of the stem. Roots 0.05–5 cm. in diameter were examined in seedlings and in full grown plants. In the youngest roots (0.05–0.15 cm.) calcium oxalate is not observed. By the time the diameter measures 0.5 cm., occasional druses appear in

the rays and in the cortex near the bases of branch roots. Druses remain infrequent, even in the largest roots. On the other hand, solitary crystals appear adjacent to phloem fibers as soon as secondary thickening begins and continue to develop in the older roots.

The dominant ergastic substance of the root is starch. In roots 0.1 cm. in diameter numerous grains are already apparent in rays and cortex. Accumulation continues, and in the older roots the ray cells are closely packed with starch. A certain amount is also present in phloem parenchyma and periderm. Fat distribution, in contrast to the condition in the stem, is sparse. A few fat idioblasts appear only in cortical and vascular parenchyma cells. Tannin sacs similar in size to those in the stem may occur in the cortex, and a few tannin-containing parenchyma cells are evident here and there.

CRYSTAL DISTRIBUTION IN SEEDLING

Seedlings in different stages of growth were examined. In the hypocotylary arch stage of development (length 2-4 cm.) the cotyledons are still inclosed within the endosperm. When the cotyledons emerge above the now straightened axis (length 5 cm.) the hypocotyl shows traces of chlorophyll and anthocyanin. As the foliage leaves expand, the full length of the hypocotyl is reached (about 7 cm.).

Calcium oxalate is only barely evident at any stage of development (figs. 14-17). While the cotyledons are below ground no calcium oxalate is observed in cotyledons, hypocotyl, root, or endosperm. As the cotyledons become green, a few druses appear at the cotyledonary node. In the older seedlings the druses increase markedly in number at the cotyledonary node and in the cotyledonary petioles. A few appear at the nodes of the growing vegetative leaves, but they are not yet obvious in the lamina.

ETIOLATED SHOOTS.—In order to compare calcium oxalate distribution in etiolated and normal shoots, plants approximately 1 m. high—and therefore with a well-developed root system and ample reserve materials—were cut off about 20 cm. above the ground. They were then protected from the light by ventilated metal containers. The temperature remained reasonably well controlled, and—even on the hottest days in mid-August—did not exceed outside temperature by more than 3° F. Other plants, pruned in the same way, were allowed to grow in the open as controls.

As a result of the severe pruning, numerous lateral shoots develop from the basal nodes. Within 6 weeks, shoots on the control plants bear four to six leaves, and removal of the screens from the darkened plants shows a similar growth of etiolated shoots. The latter are devoid of chlorophyll, the laminae are butter yellow in color and the petioles pure white. Apart from color, control and etiolated shoots differ in the usual way in area of leaves and in length of petioles and internodes (table 2).

The distribution of calcium oxalate and other ergastic substances in the green shoots does not differ from that described for the main vegetative axis. On the other hand, in etiolated shoots, while the general plan of distribution remains the same, the relative amounts of materials vary considerably. Calcium oxalate druses are apparent as usual in parenchyma of pith, cortex, vascular cylinder, nodal septa, and leaf bases, but are relatively scarce. In the leaf veins and network of veins at the junction of petiole and lamina (in contrast to the crowded condition of the normal leaf) only a very few crystals are detected. Other ergastic materials,

TABLE 2
COMPARISON OF GREEN AND ETIOLATED SHOOTS

TYPE	TOTAL LENGTH (CM.) OF TYPICAL 5-INTERNODE SHOOT WITH TERMINAL BUD AND LEAVES	LENGTH (CM.) OF TERMINAL BUD (TB) AND SUCCEEDING INTERNODES (A-E)						INTERNODES					
		TB	A	B	C	D	E		A	B	C	D	E
Green.....	3.6	0.5	0.3	0.5	0.8	0.8	0.7	Leaf size* Petiole length	2.5×3	6×7	7×9.5	6×7.5	
Etiolated....	6.1	0.7	0.4	1.9	2.5	0.5	0.8	Leaf size Petiole length	1.6×2 ¹ (still folded) 0.2	2.5×3.5 3.5	Wilted 5.5	2.5×3.2 6	4×4.5 3.2

* Length of midrib and greatest width.

such as fatty substances and tannin, do not appear to be significantly reduced in amount. In the absence of light, the starch grains of photosynthesis are naturally lacking in etiolated tissues, but the endodermis is clearly marked by starch grain content. The endodermis of the etiolated shoot is further delimited in the older internodes by the presence of typical Casparian strips, as described by PRIESTLEY (13) in *Vicia faba*. Starch grains, small but numerous, are present also in the guard cells of the stomata.

ETIOLATED SEEDLINGS (fig. 17).—Beneath the light screens, by the darkened plants, some stray seeds happened to germinate and reach the straight hypocotyl stage before dying down. These afford an interesting contrast to normal seedlings in height, color, and relative development of organs. A typical slender hypocotyl, perfectly white in color, may measure 33 cm. (more than four times the normal length). The cotyledons, butter yellow in color, remain reduced in area, actually

about one-third their normal size. The plumule remains unexpanded, while the root system appears to be normal in extent.

Sections cut at different levels indicate a scarcity of all ergastic materials. At the cotyledonary node and in the cotyledonary petioles one or two small druses are seen, but none appear in the cotyledons. Calcium oxalate is practically absent in the hypocotyl and root.

Fatty substances occur in the cotyledons and at the cotyledonary node. They occur sporadically in the epidermis of the hypocotyl and in a few cells of the transition region. Elsewhere they are scarce. Starch is extremely limited in amount. It appears in the guard cells of the cotyledons and in the endodermis in the upper hypocotyl. Elsewhere, except for small sieve-tube starch grains, it is absent.

Discussion

That crystal formation is dependent on the presence of calcium is apparent from the work of THODAY (22), and more recently OLSEN (11) has demonstrated a definite quantitative relation between calcium absorbed and oxalic acid produced and precipitated. THODAY, in the apical meristem of the succulent *Kleinia*, found a scarcity of calcium and complete absence of oxalate crystals. Both calcium and crystals appear in increasing amounts in the older parts of the plant, toward the plant base, and reach their maximum in the vascular network of the nodes. In *Ricinus* the apical meristem is likewise completely free of crystals, and their maximum distribution occurs along leaf veins and in the vascular networks of leaf centers, leaf bases, and nodes. It may be expected that microchemical analysis will indicate a lower and a higher calcium content in apical meristem and in differentiated regions, respectively.

Crystal and fat idioblasts are obvious first in the youngest internodes, in the pith. This tissue is derived from vacuolating dividing cells and is apparently homogeneous. Conditions of growth are presumably similar for all cells. It may therefore be deduced that the fate of a cell, whether destined to become a fat idioblast, a crystal idioblast, or a typical parenchyma cell, is already determined within the apical meristem. The intracellular factors of the specific anlagen emerge during meristematic division and dominate the later extra-cellular environment.

Comparison of cell sizes in the pith (of fat and druse idioblasts) indicates, in the more rapid growth of the former, an apparent conflict between the two types of metabolism, fat accumulation and calcium oxalate deposition. Within the tissue the centers of fat metabolism are more or less evenly spaced and consist of the central idioblasts—conspicuous by the immediate reduction of osmic acid—and the surrounding cells in which fatty substances are present in lesser amount.

The fatty cell groups are the first to expand and become established as metabolic units. Their continued activity implies a diversion and monopoly of materials necessary for growth, the result of which may be the dwarfing of the marginal crystal idioblasts.

The actual density of crystal formation appears to depend on the amount of ergastic substances present (9). Thus etiolated shoots and seedlings, growing at the expense of food stored in root and endosperm, respectively, are relatively poorly supplied with the sugar, etc., necessary for growth, and crystal formation is therefore slight.

In the older stem, starch metabolism—the deposition of storage starch—becomes dominant. Here, far from increasing in number at the nodes or elsewhere, calcium oxalate crystals may dissolve and disappear, and the starch-filled crystal idioblasts can be recognized only by their relative size. Quantitative macrochemical analyses are necessary to give an accurate estimate of the actual disappearance of calcium oxalate. The root, during all but the primary stages of development, is a region of starch metabolism, and calcium oxalate crystals are practically entirely absent. Conditions incidental to starch deposition are therefore unfavorable for calcium oxalate deposition and may actually cause the disappearance of fully developed crystals.

Conditions of solitary crystal growth have been occasionally discussed in connection with conditions of wall thickening (7), either in the crystal idioblast itself or in the adjacent sclerenchyma element. Wall thickening per se can hardly be considered as a potent factor in crystal environment, since crystals are somewhat exceptional in typically thick-walled xylem tissue.

In view of the generally accepted concept of pH gradient in stem tissues, the distribution of crystal types appears at first sight significant, the more so since, as PFEIFFER (12) has shown, crystal growth can be controlled in vitro by varying pH and other conditions. Preliminary experiments with pH indicators (21) were attempted with *Ricinus* but proved unsatisfactory. In further work along this line, electrometric methods are to be preferred. In *Ricinus*, however, the fact that druses occur throughout the parenchyma in general, while solitary crystals are restricted to the “relatively alkaline” secondary phloem, appears to indicate a measure of specificity in pH conditions. This specificity is not maintained in other plants, however, since solitary crystals appear abundantly in the presumably “relatively acid” xylem fibers of *Prosopis*, the desert mesquite (19).

Summary

1. The development of calcium oxalate crystals and their distribution in the mature green plant, in etiolated shoots, in green and in etiolated seedlings, is examined in relation to tissue differentiation. Two types of crystals are present,

druses and solitary crystals. The former are distributed throughout parenchyma tissues in general; the latter are practically restricted to the secondary phloem, where they are deposited chiefly in vertical files of cells adjacent to the phloem fibers.

2. The development of a druse may be traced from a single crystal at the limit of visibility within a protoplasmic strand, through an aggregate or dendritic type of growth to a miniature druse, and finally to a mature crystal entirely filling the cell. Sheaths of undetermined composition may be detected around very small crystals about 3μ in diameter. The sheaths of older druses consist of cellulose and are occasionally partly suberized. They are attached by stalks to the cell wall. Solitary crystals are also surrounded by cellulose sheaths.

3. Crystal idioblasts grow more slowly than adjacent parenchyma cells, and intercellular spaces appear around them in the youngest internodes. In contrast to this, fat idioblasts expand rapidly, maintain contact with surrounding cells, and intercellular spaces do not arise until later.

4. Crystals develop in the growing shoot about the level of the third node, and they rapidly increase in number in the differentiating primary stem. They reach their maximum concentration in the vascular networks of the nodes and leaf blade centers and along the leaf veins. Crystals are also distributed more or less regularly throughout the pith and are present also in the cortex and vascular parenchyma of the stem and of the petiole. They occur occasionally in the lamina and are frequent in the caducous stipular sheath. This greatest density of crystal deposition coincides with the maximum distribution of fat idioblasts and of sugar.

5. When secondary thickening is established in the stem, druses become masked by starch grain deposition in the pith and occasionally elsewhere. In many cases the crystals are dissolved and leave behind them the empty crystal sheath. At the same time starch accumulates and may completely fill the former crystal idioblast.

6. In contrast to druse behavior within the older stem, solitary crystals appear abundantly in vertical files of parenchyma cells adjacent to the phloem fibers in the secondary phloem.

7. In the root, except in the cortex near the origin of secondary roots, druses are practically absent. Solitary crystals, however, occur in the secondary phloem.

8. Druses are present in the pith of the young inflorescence axis approximately as in the stem. Since nodal vascular networks are absent, however, the crystals do not equal those of the shoot in number. As the seeds develop, starch deposition in the fruiting axis is extremely heavy; here, as in the older stem, druses may dissolve and disappear.

9. In the fruit wall, calcium oxalate crystals are not abundant at any stage of

development. In the seed, one or two small crystals may appear near the caruncle but do not occur elsewhere.

10. In the etiolated shoot, crystal distribution is similar to that in the green, but the number of crystals is markedly less.

11. In green seedlings, crystal development is slight, a few at the cotyledonary and first plumular nodes, rarely in the parenchyma of the hypocotyl, and occasionally in the cortex of the root.

12. In etiolated seedlings, crystals are practically absent. A few very small druses may be detected at the cotyledonary node. They are absent throughout the hypocotyl, and the plumular node does not develop.

13. Crystal distribution is discussed in relation to various factors, such as: calcium supply; tissue metabolism as indicated by the presence of fat, sugar, and starch; cell-wall thickening; hydrogen-ion concentration.

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LITERATURE CITED

1. ALDABA, V. C., Structure and development of the cell wall in plants. Amer. Jour. Bot. 14:16-24. 1927.
2. ALEXANDROV, W. G., and TIMOFEEV, A. S., Über die Lösung des kristallinen Calciumoxalats. Bot. Archiv. 15:278-280. 1926.
3. BRUNZEMA, D., Entwicklung der Kalziumoxalatzellen mit besonderen Berücksichtigung der officinellen Pflanzen. Archiv. Pharm. 266:86-103. 1928.
4. ESAU, K., Ontogeny and structure of the phloem of tobacco. Hilgardia 11:343-406. 1938.
5. FREY, A., Calcium oxalat-Monohydrat und Trihydrat. Handb. Pflanzen. Band III/1a: 82-101. 1929.
6. GRIS, A., Le développement de la graine du *Ricin*. Ann. Sci. Nat. Bot. 15:5-9. 1861.
7. KONSTANTY, E. C., Über die Entstehung der Kristallzellreihen mit besonderer Berücksichtigung der Drogenpflanzen. Bot. Archiv. 15:131-186. 1926.
8. MEYER, A., Analyse der Zelle. Jena. 1920.
9. MÜLLER, W., Über die Abhängigkeit der Kalkoxalatbildung in der Pflanze von den Ernährungsbedingungen. Bot. Centralbl. Beih. 39:321-351. 1922.
10. NETOLITSKY, F., Die Kieselskörper. Die Kalksalze als Zellinhaltskörper. Handb. Pflanzen. Band III/1a: 1-78. 1929.
11. OLSEN, C., Absorption of calcium and formation of oxalic acid in higher green plants. Compt. Rend. Trav. du Laborat. Carlsberg 23:101-123. 1939.
12. PFEIFFER, H., Über die Wasserstoffionen Konzentration [H.] als Determinationsfaktor physiologischer Gewebegeschehen in der sekundären Rinde der Pflanzen. New Phytol. 24:65-98. 1925.
13. PRIESTLEY, J. H., On the anatomy of etiolated plants. New Phytol. 25:145-170. 1926.
14. ———, Cell growth and cell division in the shoot of the flowering plant. New Phytol. 28: 54-81. 1929.
15. PRIESTLEY, J. H., and SCOTT, LORNA, Vascular anatomy of *Helianthus annuus*. Proc. Leeds Philos. Soc. (Sci. Sect.) 3: part III. 159-173. 1936.

16. REYNOLDS, M., The development of the node in *Ricinus communis*. Unpublished thesis.
17. ROSANOFF, S., Über die Krystalldrüsen im Marke von *Kerria japonica* D.C. und *Ricinus communis*. Bot. Zeitg. 23:329-330. 1865.
18. SCOTT, FLORA M., Differentiation of spiral vessels in *Ricinus communis*. BOT. GAZ. 99:69-79. 1937.
19. ———, Unpublished data.
20. SCOTT, FLORA M., and SHARSMITH, H. M., The transition region in the seedling of *Ricinus communis*. Amer. Jour. Bot. 20:176-187. 1933.
21. SMALL, J., Hydrogen ion concentration in plant cells and tissues. Protoplasma-monographien. Berlin. 1929.
22. THODAY, D., and EVANS, H., Studies in growth and differentiation. III. The distribution of calcium and phosphate in the tissues of *Kleinhia articulata* and some other plants. Ann. Bot. 46:781-806. 1932.
23. WAKKER, J. H., Über die Inhaltskörper der Pflanzenzelle. Jahr. Wiss. Bot. 19:423-496. 1888.
24. WITTLIN, J., Über die Bildung der Kalkoxalat-Taschen. Bot. Centralbl. 17:33-41; 65-73; 97-101; 129-133. 1896.

CYTOLOGICAL AND TAXONOMIC STUDIES IN THE GENUS BRODIAEA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 530

MADELINE PALMER BURBANCK

(WITH TWENTY-NINE FIGURES)

Introduction

Brodiaea is the genus name commonly applied to a group of liliaceous plants which are widespread in the Pacific coast region of western North America. Certain plants native to South America have been included in the genus in the past, but it is probable that the unjointed pedicels and other characters of the South American plants justify their assignment to a different group. The genus *Brodiaea* ranges from British Columbia in the north to Lower California in the south, being especially common on the lower slopes of the Cascade and Sierra Nevada Mountains. Certain species extend as far eastward as Montana, Wyoming, Utah, Nevada, and Arizona. The plants produce seeds but in the majority of cases are more effectively propagated by offsets from the underground corms.

The twenty-five lots of plants, both species and named varieties of species, used in this study were all received under the genus name *Brodiaea*. Although this generic name had been most commonly applied to this group of plants, various subdivisions have been given in the past and again revived in recent publications by HOOVER (4, 5, 6, 7). In the first paper of this series, HOOVER summarizes the problem of synonymy in the genus, and it is not necessary to discuss the subject at length here. JEPSON (8) recognizes the genus *Brodiaea*, which he divides into three subgenera, *Triteleia*, *Hookera*, and *Dichelostemma*. ABRAMS (1) names six genera (*Calliprora*, *Hesperoscordum*, *Triteleia*, *Hookera*, *Dichelostemma*, and *Brevortia*) to cover essentially the same plant material. More recently HOOVER's reports (4, 5, 6, 7), based on extensive field work in the western states, present morphological characters which he considers sufficiently distinct to justify a division of the plants into four genera. These are essentially the same groups as those recognized by JEPSON, with the addition of a new genus, *Triteleiopsis*. Under International Rules the reorganized groups become *Triteleia*, *Dichelostemma*, and *Brodiaea*. Since the plants used in this study bear out HOOVER's classification with respect to morphological characters and cytological observations, the generic and specific names he gives are, for the most part, used in this paper. Table 1 gives the synonymy. Species of *Brodiaea*, *Dichelostemma*, and *Triteleia* were studied, but plants of the monotypic genus *Triteleiopsis* were not available.

Brodiaea has previously been investigated cytologically by only three workers. In a list of plants possessing 10-12 unequal sized chromosomes, MÜLLER (10) in-

TABLE 1
SYNONYMY OF PLANT MATERIAL WITH EQUIVALENT REPORTED
AND OBSERVED CHROMOSOME NUMBERS

AS NAMED BY HOOVER	AS NAMED WHEN RECEIVED (PURDY)	CHROMOSOME NUMBERS		
		OBSERVED		JOHANSEN
		2N	N	
<i>Brodiaea</i>	<i>Brodiaea</i>			
<i>Coronariae</i>				
<i>B. elegans</i>	<i>B. grandiflora</i>	32	16
<i>B. coronaria</i>	<i>B. grandiflora</i> (Oregon)	42	21	2n = 42
<i>Stellares</i>				n = 21
<i>B. minor</i>	<i>B. purdyi</i>	32	16
<i>B. minor</i> var. <i>nana</i>	<i>B. minor</i>	12	6	2n = 14
<i>B. stellaris</i>	<i>B. stellaris</i>	12	6 6*	2n = 12
<i>Appendiculatae</i>				
<i>B. californica</i>	<i>B. californica</i> (Blue)	12	6	2n = 10
<i>B. californica</i>	<i>B. californica</i> (Lilac Pink)	12	6	2n = 10
<i>Dichelostemma</i>				
<i>D. pulchellum</i>	<i>B. capitata</i>	18	2n = 72
.....	<i>B. capitata</i> var. <i>multiflora</i>	18
<i>D. congestum</i>	<i>B. pulchella</i>	36	2n = 36
<i>D. volubile</i>	<i>B. volubilis</i>	18	9	2n = 36
<i>D. ida-maia</i>	<i>B. coccinea</i>	48	n = 20(ca.)
<i>Triteleia</i>				
<i>Eutriteleia</i>				
<i>T. grandiflora</i>	<i>B. douglasii</i>	32
<i>T. peduncularis</i>	<i>B. eastwoodii</i>	14
.....	<i>B. peduncularis</i>	28
<i>T. laxa</i>	<i>B. laxa</i>	28(30)	14 14*
<i>T. laxa</i>	<i>B. laxa</i> (Blue King)	42
<i>T. laxa</i>	<i>B. candida</i>	48
<i>T. crocea</i>	<i>B. crocea</i>	16
<i>Calliprora</i>				
<i>T. ixioides</i> var. <i>scabra</i>	<i>B. ixioides</i> var. <i>splendens</i>	10(11)	5(6) 5*
<i>T. ixioides</i> var. <i>analina</i>	<i>B. ixioides</i> var. <i>erecta</i>	50
<i>Hesperoscordum</i>				
<i>T. hyacinthina</i>	<i>B. lactea</i>	28
<i>T. hyacinthina</i>	<i>B. lactea</i> (Oregon)	28
<i>T. hendersoni</i>	<i>B. hendersoni</i>	32	16*
<i>T. bridgesii</i>	<i>B. bridgesii</i>	16

* Microspore counts.

cluded an unnamed species of *Triteleia*. JOHANSEN (9) worked with four species of *Hookera* (*Brodiaea*), four of *Dichelostemma*, and one of *Brevoortia* (*D. ida-maia*). The chromosome numbers he obtained are given in table 1, and in addition to those listed, he observed 30 somatic chromosomes in *D. multiflorum*. SMITH (12) reported the presence of chromosome rings and end-to-end attachment of more

than 2 chromosomes at microsporogenesis in *B. lactea*. Counts revealed about 21-24 pairs of chromosomes.

It is hoped that the present study will help to clarify relationships within the large group *Brodiaea*, through chromosome counts of approximately half the plants included in the genera *Brodiaea*, *Dichelostemma*, and *Trileia*.

Material and methods

Plants of the twenty-four species and varieties used in this study were obtained from Mr. CARL PURDY, Ukiah, California, with the exception of corms of *B. coronaria*, which were received from Dr. FRANK H. SMITH, Corvallis, Oregon. Additional material of *B. lactea* (*T. hyacinthina*) was received from Dr. D. P. ROGERS of Corvallis, Oregon.

The corms were potted in sandy loam when received in the autumn. Four or five corms of each lot were kept in a cool greenhouse and used for root-tip material. Root tips were cut off as they emerged and were fixed in La Cour's 2 BE. The material was run up through the usual schedule of alcohols, chloroform and alcohol, and imbedded in paraffin. The root tips were cut transversely at 25 μ . The gentian violet-iodine method of staining, with—in some cases—a picric acid modification (13), was used for the root-tip sections. Immediately after being potted, the corms not used for root tips were placed in a coldframe, where they remained for several months during freezing weather. On being brought into a cool greenhouse many of the plants produced flower buds. Anther smears were made of appropriate stages of meiosis, both by the aceto-carmin method and by fixation of smeared anthers in Navashin's solution followed by staining with gentian violet-iodine. A few smears were also secured which showed the mitotic division of the microspore nucleus following meiosis.

As far as possible, some flower buds of each lot of corms were allowed to mature and the flowers used for a check on the identification of the plants. Unless stated to the contrary, fresh and pressed flowers were used for identification of all plant material. The descriptions given by ABRAMS (1), JEPSON (8), and HOOVER (5, 6, 7) were most frequently used, but references to the original descriptions were made occasionally.

A few selfings and cross pollinations were made with plants which bloomed profusely. Seeds were collected from five selfings and from one cross pollination. Several other pollinations produced negative results. No attempt has been made as yet to germinate the seeds obtained.

Unless otherwise stated, all drawings were made at table level with the aid of a camera lucida, using a 15 \times compensating ocular and a 90 \times apochromatic objective, n.a. 1.40, with a yellow-green filter. The magnification is approximately 2000 \times .

Identification of plant material and cytological observations

BRODIAEA

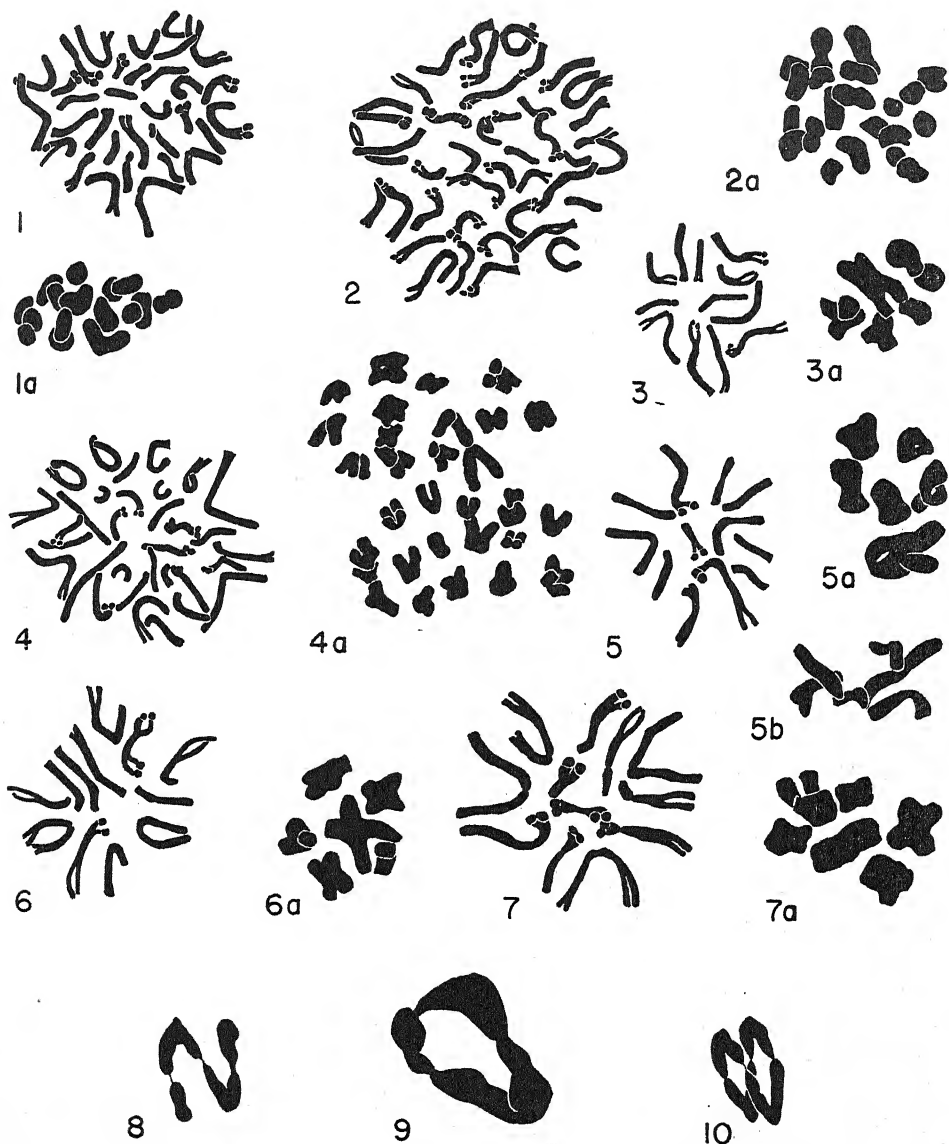
Within this genus HOOVER (5) recognizes four sections: Coronariae, Stellares, Appendiculatae, and Filifoliae. It is probable that two species of the Coronariae were employed in this study. The material received from PURDY under the name *B. grandiflora* was identified as *B. elegans* as described by HOOVER. The staminodia were acute, blunt, or irregularly three toothed, and always shorter than the stamens. The diploid chromosome number of this species is 32 (fig. 1), and 16 chromosomes (fig. 1a) were distinguishable in each early telophase I group. One chain of 4 chromosomes was seen at metaphase I (fig. 8). A few plants were selfed and two capsules of seeds obtained.

When the *B. grandiflora* material from Oregon bloomed at the same time as did the plants from PURDY, slight differences in gross morphology of the flowers could be detected. The tube of the Oregon flower was about 1 mm. longer than that of *B. elegans* and somewhat campanulate rather than funnelform; the segments were approximately the same length as those of *B. elegans* or slightly shorter and 1-2 mm. narrower; the staminodia were erect, not involute, tapered to an acute tip, and were the same length as the stamens; the anthers were 7 mm. in length as compared with 8-9 mm. in the material from PURDY. The leaves of the Oregon material were 2 mm. in diameter and were round or only slightly flattened, while those of *B. elegans* were 2-6 mm. wide and flattened. Chromosome counts of the Oregon material show a diploid number of 42 (fig. 2) and 21 chromosomes (fig. 2a) at metaphase II. Bridging was frequently observed in anaphase and telophase of meiosis I.

Although the Oregon material bloomed sparingly, the consistent flower differences, especially the relative lengths of stamens and staminodia and the distinctive chromosome number, suggest that the material is not *B. elegans*, which it resembles, but rather *B. coronaria* or a form of this species. The staminodia did not form a perfect "corona" but were not so far distant from the center of the flower as were those of the California material. A note from Dr. SMITH during the course of this investigation stated that the material which he sent as *B. glandiflora* had been identified as *B. coronaria* by Prof. HELEN M. GILKEY.

In the Stellares section, both root-tip and anther material of *B. minor* var. *nana* and *B. stellaris* showed that $2n=12$ (figs. 3, 5) and $n=6$ (figs. 3a, 5a). A metaphase figure of the microspore division was counted and verified the count of $n=6$ in *B. stellaris* (fig. 5b).

Corms were received from PURDY under the name *B. purdyi*. JEPSON (8) does not mention this name, but both ABRAMS (1) and HOOVER (5) give *Hookera purdyi* and *Brodiaea purdyi* as synonyms for *Brodiaea* (*Hookera*) *minor*. Since certain



FIGS. 1-10.—Fig. 1, *B. elegans*, root-tip metaphase. Fig. 1a, same, early telophase I. Fig. 2, *B. coronaria*, root-tip metaphase. Fig. 2a, same, metaphase II. Fig. 3, *B. minor* var. *nana*, root-tip metaphase. Fig. 3a, same, metaphase I. Fig. 4, *B. purdyi*, root-tip metaphase. Fig. 4a, same, anaphase I. Fig. 5, *B. stellaris*, root-tip metaphase. Fig. 5a, same, metaphase I. Fig. 5b, same, microspore division. Fig. 6, *B. californica* (Blue), root-tip metaphase. Fig. 6a, same, metaphase I. Fig. 7, *B. californica* (Lilac Pink), root-tip metaphase. Fig. 7a, same, metaphase I. Figs. 8-10, associations of 4 chromosomes at metaphase I. Fig. 8, *B. elegans*. Fig. 9, *T. hyacinthina* (aceto-carmin smear). Fig. 10, *B. purdyi*.

floral parts of PURDY'S *B. purdyi* were larger than the measurements given by HOOVER for *B. minor*, some doubt existed as to the correct names for the plants under investigation. The differences to be enumerated later leave no doubt that two distinct forms are involved. Since HOOVER separates *B. minor* var. *nana* from the species on the basis of smaller size without giving a complete set of measurements, the name is used here for the smaller plants. Although the other, larger plant is probably HOOVER'S *B. minor*, the name *B. purdyi* is retained in this discussion as an indication that the plants did not conform in every respect to any description given by HOOVER.

In vegetative characters *B. purdyi*, in contrast to *B. minor* var. *nana*, showed more vigorous growth with longer and broader leaves and usually more than one scape from a single corm. Significant differences in length of plant parts are given in table 2. In addition, the segments of *B. purdyi* recurved soon after the flower

TABLE 2
MEASURED DIFFERENCES BETWEEN *B. PURDYI* AND
B. MINOR VAR. *NANA*

PLANT PART	<i>B. PURDYI</i>	<i>B. MINOR</i> VAR. <i>NANA</i>
Scape.....	3-12 cm. (av. 8-9 cm.)	3-13 cm. (av. 6 cm.)
Tube.....	7-8 mm.	8-9 mm.
Segments.....	16-19 mm.	9-12 mm.
Staminodia.....	11 mm.	6-7 mm.
Anthers.....	6 mm.	4 mm.

opened, while those of *B. minor* var. *nana* did not. With increasing age the flowers of *B. purdyi* faded to a bluish violet, with a prominent, dark midvein, while those of *B. minor* var. *nana* darkened and shriveled.

Root-tip cells of *B. purdyi* contain 32 chromosomes (fig. 4). At meiosis I and II the reduced number of 16 (fig. 4a) can be observed. Occasionally there are associations of 3 or 4 chromosomes and one ring of 4 was observed (fig. 10). One plant set seed after being selfed.

The only other species of *Brodiaea* studied was *B. californica*. PURDY offers two color forms of this species, "Lilac Pink" and "Blue." Under greenhouse conditions these color differences were not always noticeable. The leaves of the Lilac Pink plants tended to be wider than those of the Blue. For both lots the mitotic chromosome number was 12 (figs. 6, 7) and the meiotic number 6 (figs. 6a, 7a). At both stages the chromosomes of *B. californica* Lilac Pink were slightly larger than those of the blue form. One of the corms received as *B. californica* Lilac Pink produced white flowers, but no irregularities of chromosome structure or behavior were observed at meiosis.

DICHELOSTEMMA

Of the six species and one variety listed by HOOVER (6) in this genus, chromosome counts were made of four species and one variety. Neither *B. capitata* nor *B. capitata* var. *multiflora*, as received from PURDY, flowered in Chicago. According to synonymy, *B. capitata* is *D. pulchellum*, but a corresponding synonym for the variety "*multiflora*" was not found. In both plants $2n=18$ (figs. 11, 12).

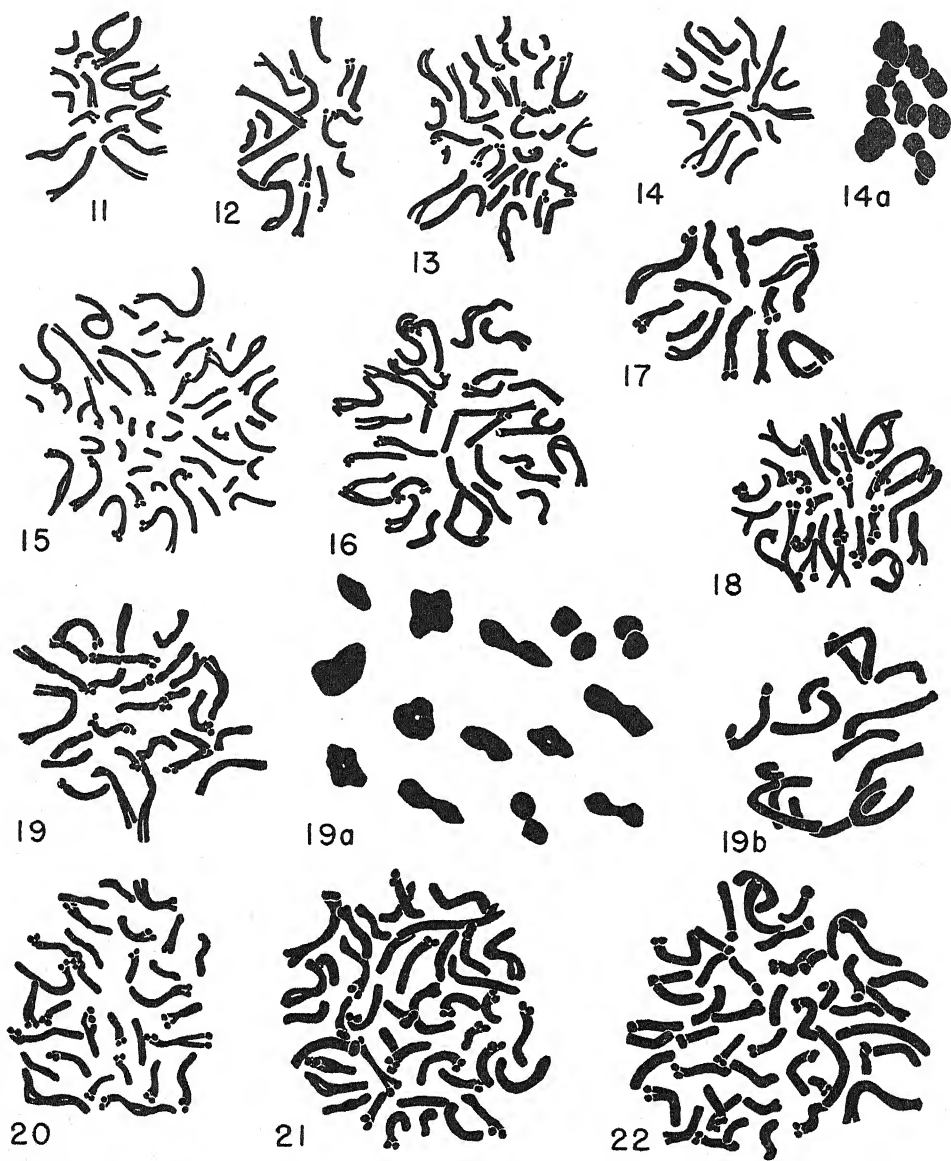
D. congestum has a diploid number of 36 (fig. 13). The few meiotic stages observed did not give a clear picture of the chromosomes, but the number of bivalents appeared to be approximately 18. *D. volubile* had a diploid number of 18 (fig. 14), and 9 bivalents (fig. 14a) were seen at metaphase I. No seeds were set following selfing. *D. ida-maia* has a diploid number of 48 (fig. 15). The large number of chromosomes made it difficult to distinguish individual bivalents at meiosis I, but at metaphase II approximately 24 chromosomes could be seen. One capsule of seed was collected following pollination of *D. volubile* with *D. ida-maia* pollen.

TRITELEIA

On the basis of the attachment and length of the anther filaments, HOOVER (7) divides this genus into three sections, Eutriteleia, Calliprora, and Hesperoscordum. First among the plants in the section Eutriteleia, which have the stamens attached alternately in two rows on the perianth, is the type species of the genus *T. grandiflora*. The somatic chromosome number is 32 (fig. 16). Although a few flowers were produced which made it possible to identify the plants, there was not sufficient material for meiotic counts, since the corms tended to split into smaller units rather than to send up flowering scapes.

The second species of the Eutriteleia section is *T. peduncularis*. Some confusion exists about two distinct plants which have been called by this name. According to PURDY (private correspondence with the writer), the plants which he has under the name *B. eastwoodii* have been identified by western taxonomists as *B. peduncularis*. The plants which he carries as *B. peduncularis* are then without a name. This matter will be discussed in greater detail later. Since the plants did not flower, PURDY's determination of two morphologically distinct plants is accepted, and according to HOOVER's nomenclature, *B. eastwoodii* is *T. peduncularis*, and *B. peduncularis* is unnamed. Examination of root-tip material reveals that for *T. peduncularis* $2n=14$ (fig. 17), and in the unnamed species $2n=28$ (fig. 18). The leaves of *T. peduncularis* were unique in that the midrib region was triangularly thickened. Between the acute angle of the keel and the upper surface of the leaf there was a space, slightly pithy, which in cross section measured about 2 mm. on each of the three sides.

According to HOOVER (private correspondence and 7), *T. laxa*, as he recognizes it, contains several forms, but no clear-cut separation can be made on the basis of



FIGS. 11-22.—Fig. 11, *D. pulchellum*, root-tip metaphase. Fig. 12, *D. pulchellum* var. "multiflora," same. Fig. 13, *D. congestum*, same. Fig. 14, *D. volubile*, same. Fig. 14a, *D. volubile*, metaphase I. Fig. 15, *D. ida-maia*, root-tip metaphase. Fig. 16, *T. grandiflora*, same. Fig. 17, *T. peduncularis*, same. Fig. 18, *T. unnamed*, same. Fig. 19, *T. laxa*, same. Fig. 19a, *T. laxa*, metaphase I; bivalents drawn separately. Fig. 19b, same, microspore division. Fig. 20, same, root-tip metaphase with 2 extra chromosomes. Fig. 21, *T. laxa* Blue King, root-tip metaphase. Fig. 22, *T. candida*, same.

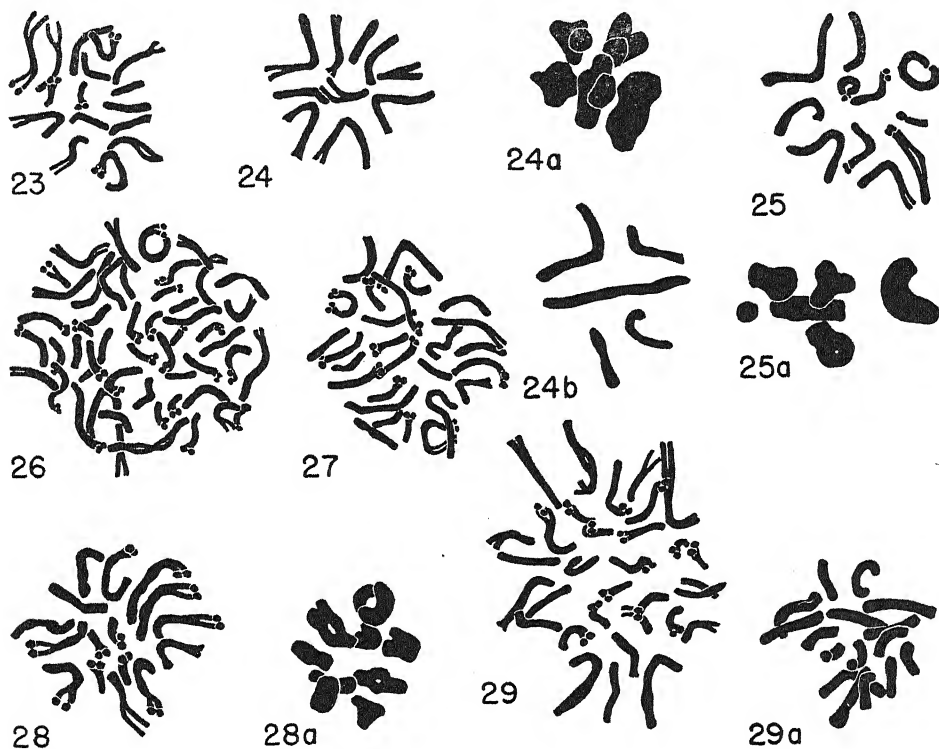
morphological characters. Material received from PURDY under the names *B. laxa*, *B. laxa* Blue King, and *B. candida* should all be called *T. laxa*, according to HOOVER. In this study, however, the species names as given by PURDY will be used with the genus name *Triteleia*, since the three plants are somewhat distinct taxonomically and cytologically. As grown in the greenhouse here, *T. laxa* Blue King usually had slightly broader leaves, longer scapes, and flowers of a deeper lavender-blue than did *T. laxa*. The material was rather variable, especially with regard to the horizontal or upright orientation of the flowers, the location of the pistils along the lower side of the flowers or in a central position, and blue or white coloration of the stamens and pollen. *T. laxa* shows $2n=28$ (fig. 19) in root tip and $n=14$ (fig. 19a) at metaphase of meiosis I and the microspore division (fig. 19b). One plant contained 30 chromosomes (fig. 20) in root-tip cells. The root-tip material of *T. laxa* Blue King shows $2n=42$ (fig. 21). The massing of the large number made accurate counting of meiotic chromosomes impossible, but in a few cells two groups of approximately 21 chromosomes each could be seen at anaphase I. At diakinesis there appeared to be about 19–23 bivalents and 3–5 univalents, with very few chiasmata evident. The haploid number of 14 was seen at meiosis in at least one plant labeled *T. laxa* Blue King and in a microspore division figure from a similar plant where the expected number would be 21. Enlargement of the ovaries followed self-pollination in both *T. laxa* and *T. laxa* Blue King, but no seeds were produced.

The plants of *T. candida* differ from those of *T. laxa* in several respects. The flowers were produced 1–2 months earlier than those of *T. laxa*; they were slightly larger, and the leaves were wider and a more yellow green, with a succulent appearance. This was not caused by some local difference in microclimate because the plants were all treated in the same way, and the differences were apparent both winters that the plants were grown. The morphological characters of *T. candida* flowers were essentially the same as those of *T. laxa*, except for the smaller size of the anthers. PURDY (private communication) maintains that *T. candida* and *T. laxa* can be distinguished by the angled pedicels and horizontal flowers of *T. candida* and by the straight pedicels of *T. laxa*, which are never angled at the base of the tube. Since this information was received from PURDY after the *T. candida* flowers had ceased blooming, fresh material could not be examined, and pressed specimens were unsatisfactory on this point. As already stated, however, some flowers of both *T. laxa* and *T. laxa* Blue King formed an angle with the pedicel, so the distinction does not always hold true.

In *T. candida* $2n=48$ (fig. 22). Again meiotic counts were difficult, but about 22–24 bivalents could be seen in several cases. Some of the configurations at metaphase I suggested multiple associations of chromosomes, but the material requires further study. Two capsules of seed were collected following self-pollination.

T. crocea, another member of the *Eutriteleia* section, had a somatic number of 16 (fig. 23). Since these plants did not bloom, no meiotic counts were possible, and PURDY's determination of the species was accepted.

The *Calliprora* section includes those plants in which the anther filaments are alternately long and short but all attached at the same level. The varieties of



FIGS. 23-29.—Fig. 23, *T. crocea*, root-tip metaphase. Fig. 24, *T. ixioides scabra*, same. Fig. 24a, *T. ixioides scabra*, metaphase I. Fig. 24b, same, microspore division (acetocarmine smear). Fig. 25, same, root-tip metaphase with 1 extra chromosome. Fig. 25a, same, metaphase I with fragment. Fig. 26, *T. ixioides* var. *analina*, root-tip metaphase. Fig. 27, *T. hyacinthina*, same. Fig. 28, *T. bridgesii*, same. Fig. 28a, *T. bridgesii*, metaphase I. Fig. 29, *T. hendersoni*, root-tip metaphase. Fig. 29a, same, microspore division.

B. ixioides received from PURDY correspond with the two varieties of *T. ixioides* which HOOVER (7) places in this group, *T. ixioides* var. *scabra* and *T. ixioides* var. *analina*. Three of the four plants from which root tips were collected showed 10 somatic chromosomes (fig. 24) in *T. ixioides* var. *scabra*. The fourth plant had 11 chromosomes (fig. 25), which exhibited greater extremes in length than did those of the "normal" complement of 10. Meiotic counts from two out of about twelve plants examined revealed an extra chromosome or chromosome fragment at meta-

phase I (fig. 25a) in addition to the normal five pairs (fig. 24a) characteristic of the majority of plants. The extra chromatic material was in the form of a small circular mass. It was never seen associated with a bivalent and frequently was at some distance from the group of bivalents. If present in a single cell, the fragment was present in all cells on the slide in which individual chromosomes or bivalents were distinguishable. At anaphase I and telophase I the fragment appeared capable of going undisturbed to one of the daughter nuclei. Frequently the fragment became isolated in the cytoplasm or remained between the two masses of cytoplasm which rounded up around each nucleus preliminary to the formation of a cell wall between the daughter cells. None of the material obtained showed the fate of the fragment in meiosis II. The reduced number of five chromosomes was observed in one microspore (fig. 24b). Two plants set seed following selfing.

T. ixiooides var. *analina* has a somatic chromosome complement of 50 (fig. 26). Meiotic stages in which the chromosomes could be counted were not obtained. In a few cells there was a small circular mass of chromatin separate from the mass of metaphase I bivalents. At later stages there was very rarely a "fragment" associated with one or both of the masses of chromosomes at telophase I and metaphase II. Attempted crosses between the two varieties with *T. ixiooides* var. *analina* as the pollen parent were unsuccessful.

Chromosome counts were made of three species included in the section *Hesperoscordum*, which contains plants with equal stamens. The material of *T. hyacinthina* from Oregon was identical with that obtained from PURDY. Both had a somatic number of 28 (fig. 27). The plants did not flower profusely, and the meiotic material suggests 14 bivalents at metaphase I and 14 in each chromosome group at metaphase II, but the determination could not be made positively. A ring of four chromosomes (fig. 9) was seen in two separate cells.

T. hendersoni has a diploid number of 32 (fig. 29). No meiotic material was obtained, but the reduced number of 16 was observed at metaphase of the microspore division (fig. 29a). In the absence of any mature flowers, the species determination of PURDY is accepted.

In *T. bridgesii* $2n = 16$ (fig. 28). Eight bivalents were observed at metaphase I (fig. 28a). Self-pollination resulted in enlarged ovaries, but no seeds were matured.

Discussion

The preceding observations indicate that the plants commonly known as Brodiaeas comprise a far from homogeneous group. Taxonomically three divisions can be recognized. Cytologically there might be postulated five groups, with basic numbers of 5, 6, 7, 8, and 9, since the diploid numbers of 10, 12, 14, 16, and 18 occur as well as multiples of these numbers. Grouped according to known chromosome numbers, the lines of the natural divisions as outlined by taxonomists would

be entirely obscured. A more fruitful—and in this case perfectly justifiable—approach to the problem is to show that each of the three genera, *Brodiaea*, *Dichelostemma*, and *Triteleia*, comprises a cytological as well as a taxonomic unit. It is unfortunate that none of the species studied have trabants or other means of ready identification of individual chromosomes. Even the centromere cannot always be located. Comparative lengths of chromosomes are helpful when cells can be found in which the chromosomes all lie in the same plane throughout their length.

Within the genus *Brodiaea*, as used in the limited sense, there are found three somatic numbers, 12, 32, and 42. Six, the gametic number of three of the six plants examined, may be considered the basic number. The similarity of the chromosomes of *B. minor* var. *nana*, *B. stellaris*, and *B. californica* is clearly seen in figures 3 and 5-7. In each plant the shortest chromosome has an approximately subterminal centromere and the longest chromosome a median constriction, indicating the position of the centromere. Likewise each of the other four chromosomes in *B. minor* var. *nana* has its counterpart in *B. stellaris* and *B. californica*. Although each chromosome of *B. californica* is larger than the similar one in the other two species, the straight progression from shortest to longest is relatively the same for all three species. This indicates a close relationship between the species.

Plants received as *B. purdyi*, $2n=32$, may be derived from a hexaploid variety of a *B. minor* var. *nana* plant containing 36 chromosomes. A theory such as the "dislocation" hypothesis of NAVASHIN (11) could account for the change from 36 to 32 somatic chromosomes. The observation of meiotic irregularities in material of *B. purdyi* lends support to a theory which involves changes in chromosome number and morphology. It is impossible to recognize the 6 basic chromosomes and determine the degree of "ploidy" by a critical examination of the somatic or meiotic cells of *B. purdyi*, but the general range of length is similar to that found in the plants in which $2n=12$.

The chromosome numbers of *B. elegans*, $2n=32$, and *B. coronaria*, $2n=42$, do not suggest a close relationship between these species and those of the Stellares and Appendiculatae, although such a relationship may have existed at one time. *B. coronaria* could be considered a heptaploid, but the number for *B. elegans* is not a multiple of 6. It is possible that polyploidy could have produced two plants with counts of 36 and 48 from plants in which $2n=12$. Subsequent additions and deletions of whole chromosomes or parts could then have brought about the evolution of the present numbers of 32 and 42. The quadrivalent association observed in *B. elegans* indicates that a reciprocal translocation has occurred. Such a hypothesis of the origin of the species does not correlate with HOOVER's statement (5) that the plants included in the Coronariae are the most primitive from the

standpoint of morphology. The facts, however, that *B. elegans* and *B. coronaria* have been confused by many taxonomists and put under one species, as may even be the case for the plants here studied, and that intermediate forms have been reported near the northern and southern limits of the range of *B. elegans*, suggest that here may be a group of plants in which chromosome number can be of aid in species delimitation. The species may be in the process of evolution through the development of ecotypes and ecospecies. The fact that *B. elegans* set seed indicates that these plants have at least reached an equilibrium which allows normal sexual reproduction, if the seeds produced are viable. The frequent occurrence of bridging in *B. coronaria* suggests that this species has not attained a similar degree of stability. Duplication of a set of 6 chromosomes cannot be identified; but as in *B. purdyi*, the general range in length and width of the chromosomes is similar to that in the other species of the genus.

JOHANSEN (9) previously reported chromosome counts for four species of *Hookera*, which is synonymous with *Brodiaea* as used by HOOVER (4). His $2n = 12$ for *B. stellaris* agrees with the present findings. His drawing of a root-tip cell of *B. californica* suggests that perhaps 2 of the longest chromosomes may in reality be 4. Such an interpretation would then give a somatic count of 12 as reported here. Conversely, 2 of the chromosomes of *B. minor* as drawn by JOHANSEN would need to be joined to 2 others to agree with the present count of 12. Such corrections are suggested on the basis of very clear mitotic and meiotic figures. The haploid and diploid numbers of 21 and 42, respectively, reported by JOHANSEN for *Hookera coronaria*, suggest that the material tentatively named *B. coronaria* in this study is indeed that species. Sufficient meiotic material was not available for JOHANSEN to observe the presence or absence of irregularities in chromosome behavior in *B. coronaria*.

Dichelostemma is the most homogeneous genus with respect to chromosome number. *D. pulchellum*, a variety of this species, and *D. volubile* each have a somatic number of 18. Nine bivalents could be clearly distinguished at metaphase I in *D. volubile*. *D. congestum*, which has 36 chromosomes, is then a tetraploid or a hexaploid, depending upon the point of view. On the basis of these four plants alone, and bearing in mind that they are assigned to a separate genus, it would be supposed that the basic number is 9 and that *D. congestum* is a tetraploid. Added to this evidence is that of the production of seed by *D. pulchellum*, which would be more usual in a diploid than in a triploid. On the other hand, *D. ida-maia*, with a number of $2n = 48$, is also included in the genus. Forty-eight is a multiple of 6 but not of 9. It has been suggested by many that, because of the peculiar type of flower, *D. ida-maia* should be assigned to a separate genus. There are, however, certain morphological similarities to the members of the genus *Dichelostemma*, and if it is true that *D. venustum* is a hybrid of *D. volubile* and

D. ida-maia, the latter would be expected to belong to the same genus rather than to a separate one. In this work several flowers of *D. volubile* were pollinated with *D. ida-maia* pollen, and two somewhat shriveled seeds were produced. Whether or not they are capable of germination has not yet been determined.

A hypothetical solution of the relationships of the plants included in *Dichelostemma* would be that far back in the beginning of the *Brodiaea* complex, 6 was the basic number. By various means one group of plants may have evolved with 9 as a secondarily basic number. Paralleling the duplication of certain chromosomes to give the diploid number of 18, an octoploid with 48 chromosomes may have arisen which in certain respects had a genetic make-up similar to that of the $2n = 18$ plant. Thus gross morphological characters might be similar and chromosome complements be sufficiently alike to allow crossing. It would be of interest to know the chromosome number of *D. venustum*.

JOHANSEN (9) counted the chromosomes of five species in this genus, one of which, *D. multiflorum*, was not obtained for this study. The reported $2n$ number of 30 might be considered to strengthen the hypothesis of 6 as a basic number. The counts of three other species by JOHANSEN agree in the case of *D. congestum* but are multiples of 18 for *D. volubile* and *D. pulchellum* (table 1). Such variations suggest the existence of polyploid races within a species which are indistinguishable in general morphology. JOHANSEN counted about 20 haploid chromosomes in meiosis in the microsporocytes of *Brevoortia* (*D. ida-maia*) and reported that this may be a plant with oscillating chromosome numbers. Although there was some difficulty in counting the chromosomes of *D. ida-maia* for this study, it was attributed to the fact that several very small chromosomes were easily obscured by the larger ones rather than to an actual variation in number. JOHANSEN mentions this striking variation in size. *D. congestum* likewise has some very small chromosomes. These short chromosomes, and the fact that all the species included in *Dichelostemma* tend to have much smaller chromosome diameters than do those of the other genera, further justify the separation of this group into a distinct genus.

Cytologically the genus *Triteleia* is distinguished by the variation in chromosome numbers among the species and the high proportion of species and varieties with meiotic irregularities. In addition, *T. ixioides* var. *scabra* and *T. laxa* plants contained individuals with chromosome numbers higher than the number in the majority of plants. This aneuploid condition is similar to that found by BEAL (2) in the Mariposa section of *Calochortus*. Somatic chromosome numbers of *Triteleia* include 10 (11), 14, 16, 28 (30), 32, 42, 48, and 50. Three groups can be recognized: (a) those with 10 (11) and the decaploid 50; (b) those with a diploid number of 14, the tetraploids with 28, and the hexaploid with 42; (c) those with a diploid number of 16 and the tetraploids with 32. The species with a diploid number of 48 could be a hexaploid in the third group or belong to a distinct group of

which the basic number was 6. The material examined gave no cytological clue as to possible relationships. Table 1 shows that the sections set forth by HOOVER (7) and the preceding three groups do not include the same plants. Further cytological and taxonomic work on the genus is needed to reconcile these differences.

No basic number for *Triteleia* can be suggested on the basis of present findings. Multiples of 7 and 8 occur in equal numbers. The presence of rings of 4 chromosomes in *T. hyacinthina*, rings of 4 and 6 chromosomes reported by SMITH (12) for *T. hyacinthina* plants with probably twice the chromosome number reported here, fragments in the two varieties of *T. ixioides*, the paucity of chiasmata and the presence of univalents in the *T. laxa* Blue King material, and the early separation of the small chromosomes at meiosis in the *T. laxa* material, all suggest that chromosome changes have played and are continuing to play a significant role in the evolution of species of *Triteleia*. No wonder it is a difficult group taxonomically. It is even possible that 6 was again the original number and other numbers have been derived from a diploid plant with 12 chromosomes. The mechanism for such changes has been outlined by NAVASHIN (11) and applied by BEAL (2) in his discussion of the Mariposa section of *Calochortus*.

The foregoing discussion of cytological findings has demonstrated that chromosome counts may be used to substantiate the division of *Brodiaea* into three genera. Each group is characterized by a separate basic number or by the absence of such a number. It is realized that the evidence is not conclusive, that on the basis of chromosome number alone, *B. elegans* and *B. coronaria* could be assigned to the *Triteleia* group or even all the plants considered a part of a single genus complex, but combined cytological and taxonomic findings indicate that the division into three groups is justified.

While the chromosome counts agree with taxonomic classification in general, this study indicates the need for taxonomic reexamination of certain species. First in *Brodiaea*, chromosome counts would indicate that *B. nana* var. *minor* is the species ($2n=12$) and that the material received as *B. purdyi* ($2n=32$) is a variety derived from the plants with a lower count. More vigorous growth as noted in *B. purdyi* is frequently associated with polyploidy. The presence of a ring of 4 chromosomes and a chain of 3 at meiosis I suggests polyploidy and chromosome rearrangement, including reciprocal translocation. HOOVER, in a private communication to the writer, suggested that the material which PURDY carries as *B. minor* may be *B. minor* var. *nana*. He further stated that EASTWOOD, who originally described *B. purdyi*, has seen the type specimen of *B. minor* in England and finds the two identical.¹ It then remains to re-examine the question of whether

¹ It is also interesting to note that the *B. purdyi* material grown here corresponds more closely with EASTWOOD's (3) original description of the species than with HOOVER's (5) description of *B. minor*. EASTWOOD gives the staminodia length as 13 mm. in contrast to 7-10 mm. and further states that the perianth segments spread widely, recurve, and possess a dark midvein.

B. minor and *B. minor* var. *nana* are a species and a variety or whether they each deserve specific rank. The difference in chromosome number lends support to the latter view, as do also the differences in the flowers as given earlier in this paper. A knowledge of the chromosome numbers of the forms reported by HOOVER (5) which are intermediate between *B. minor* and *B. minor* var. *nana* might be critical for a solution of the problem.

Taxonomic and cytological findings seem to be in agreement within the genus *Dichelostemma*. This is not so completely true in *Triteleia*. Chromosome counts may be a definite aid in the solution of the status of *B. peduncularis* and *B. eastwoodii*. The latter has never been "adequately published under existing rules of nomenclature" (HOOVER, private communication), and for that reason is not listed anywhere as a synonym by him. In the following discussion, however, *B. peduncularis* and *B. eastwoodii* will be used for the sake of clarity to designate the plants received from PURDY under those names. The diploid number of *B. eastwoodii* is 14 and that of *B. peduncularis* is 28. This suggests that the latter is a tetraploid which has arisen from *B. eastwoodii*. The greater length of peduncle reported by PURDY would further substantiate the theory. The isolated and limited habitat, supposedly inaccessible to early botanists, in which *B. peduncularis* was found by PURDY, may indicate that it is of recent origin and has not yet extended its range. Whether it was found by earlier botanists is questionable. PURDY maintains that his *B. peduncularis* more nearly resembles the plant described by WATSON than does the plant for which the description was made, PURDY's *B. eastwoodii*. In this he is supported by Mr. BAKER of Santa Rosa Junior College. Both EASTWOOD and HOOVER maintain that PURDY's *B. eastwoodii* is the true and original *B. peduncularis* as described by WATSON. PURDY suggests that perhaps type localities in some of the older collections are inaccurate, and that his *B. peduncularis* might have been collected and described as such. Later collectors might then have collected PURDY's *B. eastwoodii* from the incorrectly given type locality of *B. peduncularis* and assumed the material to be *B. peduncularis* without examining it critically. Whatever is decided concerning the names and taxonomic classification of the two plants, the chromosome numbers indicate that they are closely related and that "*B. peduncularis*" could be a tetraploid derived from "*B. eastwoodii*."²

Observations made upon the plants received from PURDY under the specific and varietal names *T. laxa*, *T. laxa* Blue King, and *T. candida* indicate that here, indeed, are two species and a variety or form of one of the species rather than a single species, *T. laxa*, as described by HOOVER. *T. laxa* has a haploid number of 14 and diploid number of 28. The observance of 30 somatic chromosomes in the

² Private communications from PURDY are the source of much of the information given in this paragraph.

roots of only one plant indicates that this number is the exception and not the rule. The presence of 42 somatic chromosomes in *T. laxa* Blue King suggests that this is a hexaploid form of the *T. laxa* material. As the plants flowered in the greenhouse, those of *T. laxa* Blue King were darker in color, the perianth segments did not spread so widely, and the leaves were slightly wider than those of *T. laxa*. In other characters, however, such as angle of attachment of the flowers, color of the stamens, and perhaps even chromosome number, the *T. laxa* Blue King plants were variable and intergraded with *T. laxa*. Such evidence indicates that *T. laxa* is a variable species, and that while chromosome counts may give a basis for separation into varieties, consistent morphological characters may not exist to make the separation possible.

Plants studied as *T. candida*, however, have both morphological and cytological characters which distinguish them from the *T. laxa* complex. The somatic number is 48, which is not an exact multiple of 7 as are the counts for *T. laxa*. The anthers of *T. candida* are only 2 mm. in length, while those of *T. laxa* are 3–4 mm. *T. candida* finished blooming before *T. laxa* had opened a single flower. The leaves of *T. candida* were yellowish green and 16 mm. wide; those of *T. laxa* were dark green and 5–10 mm. wide; those of *T. laxa* Blue King were 7–15 mm. wide. It is debatable whether "*candida*" is the correct specific name to use, since the flowers of the plant originally described as *T. candida* were white, but the need for separation from *T. laxa* is indicated.

The presence of an extra chromosome in the root-tip cells of one plant of *T. ixioides* var. *scabra* does not alter the fact that 10 is considered the typical somatic number. The fact that an extra fragment is present at meiosis lends interest to the situation. Figures 24 and 25 indicate that the increased number is not merely the result of the duplication of a single chromosome, nor could there have been a loss of a single chromosome from a complement of 12. The normal complement is made up of a pair of short chromosomes with a subterminal centromere, 4 chromosomes of medium length with submedian centromeres, and 4 long chromosomes—2 with a submedian centromere and 2 with an approximately median centromere. In the complement with an extra chromosome there are 4 long chromosomes and 4 of medium length, and in addition an extra long chromosome, a shorter than normal chromosome, and a chromosome of about the same length as the shortest in the normal complement. Such a situation could have arisen through duplication of a medium length or a small chromosome which subsequently became fragmented. One small fragment may have become attached to an end of one of the long chromosomes with a median centromere. Another fragment may have become attached to one of the shortest chromosomes. It is supposed that meiotic figures with an extra chromatin mass at metaphase I occurred in plants with a somatic number of 11, but there are no records to prove

the assumption. If the "fragment" did arise through duplication and fragmentation, it is strange that it was never observed associated at meiosis with one or both of the chromosomes of which it was originally a duplicate. Its small size is the most probable contributing cause. If the fragment possesses a centromere, it could be carried regularly through the process of sexual reproduction and be perpetuated. Also through vegetative propagation an irregularity of chromosome number could be retained in ever-increasing numbers of plants, regardless of whether or not they were capable of sexual reproduction. If the change had occurred far back in the history of the species, subsequent genic changes could account for the absence of multiple associations. This explanation of the presence of a chromosome fragment is largely hypothetical, but it is an application of NAVASHIN'S dislocation hypothesis (11) and is based on the known facts that trisomic plants may arise and that parts of chromosomes may be translocated to non-homologous chromosomes.

Summary

1. The chromosome numbers of twenty-four species and varieties of *Brodiaea* have been investigated.
2. Somatic numbers of 10 (11), 12, 14, 16, 18, 28, 28 (30), 32, 36, 42, 48, and 50 chromosomes were observed. An incomplete series of corresponding gametic numbers includes 5 (6), 6, 8, 9, 14, 16, and 21.
3. Taxonomic and cytological findings seem to justify the separation of the material examined into three genera, *Brodiaea*, *Dichelostemma*, and *Triteleia*. It is suggested that the basic number for *Brodiaea* is 6, and for *Dichelostemma* the secondarily basic number 9, derived from 6; the variation of numbers in *Triteleia* is too great to postulate any one basic number.
4. The need for reconsideration of certain specific delimitations is suggested on the basis of cytological and taxonomic observations.
5. Variation of chromosome number is reported for two plants and an explanation for one of the variations offered on the basis of duplication of a chromosome and subsequent dislocation.

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LITERATURE CITED

1. ABRAMS, LEROY, An illustrated flora of the Pacific states. I. Stanford. 1923.
2. BEAL, J. M., Cytological studies in relation to the classification of the genus *Calochortus*. BOT. GAZ. 100:528-547. 1939.
3. EASTWOOD, ALICE, Descriptions of some new species of Californian plants. Proc. Cal. Acad. Sci. (II) 6:422-430. 1896.
4. HOOVER, R. F., A definition of the genus *Brodiaea*. Bull. Torr. Bot. Club 66:161-166. 1939.
5. ———, A revision of the genus *Brodiaea*. Amer. Mid. Nat. 22:551-574. 1939.
6. ———, The genus *Dichelostemma*. Amer. Mid. Nat. 24:463-476. 1940.
7. ———, A systematic study of *Trileleia*. Amer. Mid. Nat. 25:73-100. 1941.
8. JEPSON, W. L., A manual of the flowering plants of California. Berkeley. 1925.
9. JOHANSEN, D. A., The chromosomes of the California Liliaceae. I. Amer. Jour. Bot. 19: 779-783. 1932.
10. MÜLLER, H. A. C., Kernstudien an Pflanzen. Archiv. Zellforsch. 8:1-51. 1912.
11. NAVASHIN, M., The dislocation hypothesis of evolution of chromosome numbers. Zeitschr. Ind. Abst. Vererb. 63:224-231. 1932.
12. SMITH, F. H., Preliminary studies of chromosome rings in *Brodiaea lactea*. Proc. Nat. Acad. Sci. 19:605-609. 1933.
13. ———, The use of picric acid with the gram stain in plant cytology. Stain Technol. 9: 95-96. 1934.

SEROLOGICAL STUDIES OF THE ERWINEAE. II. SOFT-ROT GROUP; WITH SOME BIOCHEMICAL CONSIDERATIONS

R. P. ELROD

Introduction

The taxonomy of the soft-rot group of organisms has had the attention of many workers in phytobacteriology. The group includes four species: *Erwinia carotovora* (Jones) Holland, *E. phytophthora* (Appel) Bergey *et al.*, *E. solanisapra* (Harrison) Holland, and *E. aroideae* (Townsend) Holland. Two other little known species are sometimes included, *E. flavida* (Fawcett) Magrou and *E. erivanensis* (Kalantarian) Bergey *et al.* Much evidence has accumulated to the effect that the group is but one variable species (11, 21, 2, 8), and numerous facts have also been offered for the separation of one or more species (13, 12, 5, 10). On the basis of previous work, however, it has become increasingly clear that the group at best is a heterogeneous one, and probably not susceptible to satisfactory classification.

Both *E. aroideae* and *E. solanisapra* have been declared to be variants of either *E. carotovora* or *E. phytophthora*. LEACH (11), in his list of synonyms for the black-leg pathogen, fails to list *E. aroideae*, but apparently he considers it the same as *E. carotovora*. BONDE (2) supports this idea, which was originally proposed by HARDING and MORSE (8). The latter workers considered it to be only an anaerogenic strain of *E. carotovora*. *E. solanisapra*, on the other hand, is considered by LEACH as synonymous with *E. carotovora*. MORSE (17) likewise concluded that *Bacillus atrosepticus*, *B. melanogenes*, and *B. solanisaprus* were but one species. He was supported in this contention by JENNISON (9).

From the extensive evidence, both pro and con, there seem to stand out two significant facts in favor of separating the soft-rot group into two distinct species: (1) results of inoculating into potato plants; and (2) maltose fermentation. According to LACEY (10) and DOWSON (5), if an organism can cause a soft-rot of potato accompanied by blackening of the stems, and if it ferments maltose, it is to be considered distinct from *Bacterium carotovorum*, which does not blacken potato stems or ferment maltose. This organism DOWSON calls *Bact. phytophthorum* (Appel) *comb. nov.* LEACH has contended that stem blackening cannot be accepted as a criterion for separation, since, according to his experiments with a representative group of soft-rot organisms, there occurred varying degrees of blackening, which offered no clear-cut differentiation. He concluded that it was impossible to separate one species from another on this basis. DOWSON (4), however, confuses

the issue still further by considering *Bact. aroideae* and *Bact. carotovorum* distinct, and lists the latter as "maltose—" and the former as "maltose+."¹

It was deemed advisable to determine whether any correlation does exist between maltose+ and maltose— isolates and definite serological grouping. Before beginning the immunological studies, however, it was decided to ascertain whether or not there were any other fermentation data which might aid in either grouping all into a single species or separating into two species. At the same time, the antigenic structure of the organisms has been examined more thoroughly than heretofore. It was considered worthwhile also to ascertain the nature and specificity of extractable carbohydrate materials.

CULTURES EMPLOYED

The cultures employed² are as follows:

- 4.—*E. aroideae*, isolated from carrot, Brown University, R. P. Elrod, 1938.
- 5.—*E. aroideae*, isolated from carrot, Brown University, R. P. Elrod, 1938.
- 11.—*E. aroideae*, isolated from carrot, Brown University, R. P. Elrod, 1938.
- P4.—*E. phytophthora*, isolated from potato, Ohio State University, R. P. Elrod, 1939.
- ES.—*E. phytophthora*, isolated from *Delphinium ajacis* L., P. A. Ark, Berkeley, California, 1938.
- NZ.—*E. melonis*, isolated from turnip, E. L. Cunningham, Auckland, New Zealand, 1939.
- EA.—*E. aroideae*, isolated from pumpkin fruit, P. A. Ark, Berkeley, California, 1938.
- CA1.—*E. carotovora*, University of Chicago, G. K. K. Link, 1939.
- CA2.—*E. carotovora*, University of Chicago, G. K. K. Link, 1939.
- EP.—*E. solanisapra*, A. T. C. C. no. 4668, 1939.
- EMN.—*E. melonis*, A. T. C. C. no. 920, 1939.
- 496.—*E. phytophthora*, A. T. C. C. no. 496, 1940.
- 495.—*E. carotovora*, A. T. C. C. no. 495, 1940.
- 494.—*E. aroideae*, A. T. C. C. no. 494, 1940.
- EC.—*E. carotovora*, Ohio State University stock culture, originally from University of Texas, 1938.
- WV3.—*B. atrosepticus*, originally from United States Department of Agriculture, obtained through A. R. Stanley, University of West Virginia, 1940.

¹ Throughout this paper, maltose— refers to the failure to ferment maltose, while maltose+ indicates the formation of acid, or acid and gas.

² The writer is indebted to Drs. P. A. ARK, G. K. K. LINK, E. L. CUNNINGHAM, and A. R. STANLEY for some of the cultures employed.

WV4.—*B. dissolvens*, originally from United States Department of Agriculture, obtained from A. R. Stanley, University of West Virginia, 1940.

WV6.—*B. dissolvens*, isolated from stalk rot of sweet corn, A. R. Stanley, University of West Virginia, 1940.

All these cultures proved pathogenic on either carrot or potato slices, some to a far greater degree than others. Each organism in the list bears the name under which it was received. Several of these are now considered synonymous with one of the four accepted species. *E. melonis* (Giddings) Holland is now declared to be a synonym of *E. aroideae* (CHESTER, in BERGEY *et al.*, 1). *Bacillus dissolvens* is said to be identical with *E. carotovora* (20, 1), while *B. atrosepticus* is considered synonymous with *E. phytophthora* (11, 1).

I. Fermentation experiments

METHODS

Fermentative studies of the soft-rot group have seldom been undertaken with any completeness. Except for the more common carbohydrates, our knowledge of the fermentative abilities of these organisms has been meager. STAPP (21) made a detailed study of the fermenting capacities of many of the soft-rot organisms, but this was done before the group, as now understood, had been formulated. With this in mind, it was deemed advisable to employ as many fermentable substances as were on hand. These totaled twenty-six carbohydrates, higher alcohols, and glucosides.

Durham fermentation tubes were employed. The medium was nutrient broth to which was added enough of the fermentable substance to make a solution of 1 per cent. Brom-cresol-purple was used as an indicator. The sugars were sterilized by filtration through either a Seitz or Berkefeld filter and added aseptically to the sterile broth. Tubes were inoculated from fresh agar cultures and observed over a period of 5 weeks. The experiments were performed in triplicate.

RESULTS

Table 1 shows how active all the isolates were in utilizing the majority of the carbohydrates. Lactose, sucrose, dextrose, arabinose, galactose, inositol, levulose, mannitol, salicin, mannose, and glycerol were fermented in each instance. Erythritol, melezitose, inulin, dextrin, and dulcitol were fermented in rare cases. Sorbose and adonitol were attacked by none of the eighteen isolates. Eight cultures fermented maltose, while six broke down sorbitol.

On the criterion of gas production, the eighteen cultures formed roughly three groups: (a) those that formed only acid in any of the media (EC, 5, 11, 4, EA, EMN, WV3, and 494); (b) those that produced a small, immeasurable quantity

TABLE 1*
BIOCHEMICAL REACTIONS

ORGANISM TESTED	DEXTRROSE	SORBOSE	GALACTOSE	LEVULOSE	MANNOSE	ARABINOSE	XYLOSE	MELIBIOSE	MALTOSE	LACTOSE	SUCROSE	CELLOBIOSE	RHAMNOSE	TREHALTOSE	MELEZITOSE	RAFFINOSE	ADONITOL	SORBITOL	INOSITOL	DULCITOL	MANNITOL	ERYTHRITOL	GLYCEROL	SALICIN	AMYGDALIN	INULIN
BC.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	A	—	A	—	A	A	—	—
5.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	—	—	A	—	A	A	—	—
II.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	—	—	A	—	A	A	—	—
4.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	—	—	A	—	A	A	—	—
EA.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	—	—	A	—	A	A	—	—
EMN.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	—	—	A	—	A	A	—	—
WV3.....	A	—	A	A	A	A	A	A	—	A	A	A	A	A	—	A	—	—	—	—	A	—	A	A	—	—
494.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
NZ.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
495.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
ES.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
496.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
P4.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
EP.....	A°	—	A°	A°	A°	A°	A°	A°	—	A°	A°	A°	A°	A°	—	A°	—	—	—	—	A	—	A	A	—	—
CA1.....	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	A°	+	+	+	+	+	+	+
CA2.....	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	A°	+	+	+	+	+	+	+
WV4.....	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	A°	+	+	+	+	+	+	+
WV6.....	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	A°	+	+	+	+	+	+	+

* +, Acid and measurable gas; A°, acid and bubble of gas; A, acid only; —, no acid or gas.

of gas in some media, often only a small bubble that dissolved into the liquid (NZ, ES, 496, and 495); and (c) those that produced at least 10 per cent gas in the vial (P4, EP, CA1, CA2, WV4, and WV6). All the last group fermented maltose, while none of the acid-forming group attacked this disaccharide. Two of the intermediate group (ES and 496) also fermented maltose, with the formation of acid only, while the two other members (495 and NZ) failed to do so.

The culture NZ was a fairly recent isolate (2 months) when tested and probably never had the ability to form abundant gas. Culture ES has had a peculiar history. When first received it produced abundant gas in lactose, dextrose, and sucrose, but this property was lost while the culture was continued in stock. Experiments, however, proved that this loss of gas production did not parallel an antigenic change. Both 495 and 496 had been carried in stock for years and might possibly have lost the ability to form gas abundantly. It is probably also worthy of note that 496 was originally identified as *B. phytophthorus* by Appel, from whom it was received in 1906 by the Bureau of Plant Industry, United States Department of Agriculture. Culture 495, on the other hand, was received from L. R. Jones in 1920 by the American Type Culture Collection as a culture of *E. carotovora*. The history of the latter two organisms is interesting in view of the fact that 496 is maltose+ and 495 is maltose-.

The writer supports the contention that *E. aroideae* is synonymous with *E. carotovora*. In the present small series of fermentation tests are found every intergradation of gas production, and also evidence that a strong aerogenic organism can become almost anaerogenic. It seems improbable that a definite division can be made on the basis of gas production. STANLEY (19) recorded sixty-seven changes in the fermentative capacity of forty-three organisms in the breakdown of dextrose, lactose, and sucrose. These changes included the inability to ferment one or more of those sugars as well as changes from an aerogenic to an anaerogenic state, or vice versa.

In the maltose-fermentation experiment this substance was broken down by all the gas-producers and some of the anaerogenic strains. Two of the strains, NZ and 495, which fermented many carbohydrates with the formation of a small quantity of gas, did not ferment maltose. In subsequent experiments NZ was proved to be identical antigenically with a strain of *E. aroideae* (EA), which is a strictly anaerogenic organism. The case of 495 has already been mentioned. If it is in reality representative of the *E. carotovora* group, it would be expected not to ferment maltose (5). Most of the organisms at my disposal, however, considered to be *E. carotovora* or a synonym of it, fermented maltose.

Of all the substances used, only one other (sorbitol) seems to offer anything for satisfactory use in the way of a natural separation. As can be seen in table 1, all the abundant gas-producers fermented sorbitol, while none of the anaerogenic or

meager gas-formers broke it down. Sorbitol fermentation experiments have been previously reported in this group by STAPP (21). He obtained negative results with all his strains, which included *Bacillus phytophthorus*, *B. atrosepeticus*, *B. carotovorus*, and *B. solanisaprus*. Two organisms (ES and 496), which fermented maltose with the production of acid only, failed to attack sorbitol. But for these reactions, there was absolute agreement between the maltose and sorbitol fermentation. It is entirely possible that sorbitol fermentation is of considerable significance. This can be determined only by a more exhaustive study.

II. Serological results

Immune sera were prepared against thirteen of the eighteen cultures: four anaerogenic strains—4, EA, EMN, and 11 (all maltose—); three which produced small quantities of gas—NZ (maltose—), 496, and ES (maltose+); and all six of the gas-producing organisms—P4, EP, CA1, CA2, WV4, and WV6 (all maltose+).

The results of the cross-agglutination tests are found in table 2. It is evident that there was a fair degree of cross-agglutination throughout the whole group, since there was a total of forty reactions out of a possible two hundred and twenty-one, or 18.1 per cent. This is exclusive of the homologous reactions. Only those reactions which attained at least a titer of 1:40 were considered. The average homologous titer of the thirteen sera employed was 22,110, while the forty heterologous reactions had an average titer of 2950. The titers of the cross-reactions ranged between 80 and 20,480. Normal sera drawn in every case failed to agglutinate any of the organisms in a dilution of 1:20 or greater.

If, however, the maltose+ and the maltose— organisms are considered separately, a far higher degree of correlation is obtained. The five sera prepared against the non-maltose fermenting organisms agglutinated 33.3 per cent (fifteen of a possible forty-five) of the maltose— group. The average homologous titer of these sera was 13,312, while the fifteen heterologous titers averaged 2251. On the other hand, the eight sera prepared against maltose+ organisms produced 28.6 per cent (sixteen of a possible fifty-six) cross-reactions with the maltose fermentors. The average homologous titer in this case was 27,885 and the heterologous average was 4165.

Although the percentage of cross-reaction was higher in the maltose— group, three organisms, WV3, 494, and 495, failed to react with any of the sera. The percentage of cross-reactions would doubtless have been lower had sera been prepared against one or more of these organisms. The maltose+ organisms all showed some degree of cross-agglutination within that group.

Probably just as significant as the fairly high percentage of cross-reaction within the fermentative groups, if not more so, was the very small amount of cross-agglutination found among them. There were but nine reactions out of a possible

one hundred and twenty, or only 7.5 per cent. Hence the bulk of the 18.1 per cent cross-reaction occurred within the maltose+ and maltose- groups. Four of the nine crosses were due to the borderline organism, ES, which produces only acid in maltose and serologically is extremely close to two members of the maltose- group. Subsequent adsorption experiments have shown EA and NZ to be identical and ES to contain an antigenic component common to these two.

TABLE 2*
RESULTS OF CROSS-AGGLUTINATION EXPERIMENTS

ORGANISM AGGLUTINATED	IMMUNE SERA PREPARED AGAINST												
	MALTOSE -					MALTOSE +							
	4	EA	EMN	11	NZ	ES	496	P ₄	EP	CA ₁	CA ₂	WV ₄	WV ₆
4.....	20480	80	320	10240	—	—	—	160	—	—	—	—	—
EC.....	—†	—	—	—	80	—	—	—	—	—	—	—	—
EA.....	—	10240	—	80	10240	5120	—	—	—	—	—	—	—
5.....	640	—	160	640	—	—	—	—	—	—	—	—	—
EMN.....	80	—	5120	160	—	—	160	—	—	—	—	—	—
11.....	320	160	320	20480	—	—	—	160	—	—	—	—	—
WV ₃	—	—	—	—	—	—	—	—	—	—	—	—	—
494.....	—	—	—	—	—	—	—	—	—	—	—	—	—
NZ.....	—	10240	—	—	10240	5120	—	—	—	—	—	—	—
495.....	—	—	—	—	—	—	—	—	—	—	—	—	—
ES.....	—	640	—	—	5120	40960	—	—	—	—	—	—	—
496.....	—	—	—	—	—	—	5120	—	—	—	—	5120	—
P ₄	640	—	—	320	—	640	—	20480	160	2560	80	—	—
EP.....	—	—	—	—	—	320	—	—	40960	—	—	5120	20480
CA ₁	—	—	—	—	—	160	—	5120	—	20480	1280	—	—
WV ₄	—	—	—	—	—	—	2560	—	5120	—	—	10240	—
WV ₆	—	—	—	—	—	—	—	—	10240	—	—	—	81920

* Normal serum and saline controls negative. All titers referred to as their reciprocals.

† —, less than 1:40.

Adsorption experiments were conducted in the majority of the cases in which cross-agglutination occurred. The numerous experiments were not informative. It could be definitely shown that for the most part the various agglutinative groups did possess common factors, which manifested themselves on adsorption by causing a reduction of the homologous titer. By means of these experiments it was possible to identify twenty-two different flagellar components in the eighteen organisms. Their pattern, however, offered no hope of forming definite serological groups.

During the course of agglutination and agglutinin-adsorption investigations, a situation arose that was felt to need further study. The organism EP has been shown to be virtually analogous to CA1, CA2, and P4 from a biochemical stand-

point. To be sure there were certain differences, but these apparently were not significant. Serologically, EP showed little relationship to these three organisms. Only in the case of P₄, which agglutinated to a titer of 160 in EP antiserum, was there evidence of cross-reaction. The reason for this unilateral expression was difficult to determine.

With this in mind, adsorption tests were carried out with EP on P₄ antiserum. As can be seen in table 3, all the cross-reacting agglutinins were removed and the homologous titer was reduced from 20,480 to 1280. Repeated adsorptions failed to reduce this latter figure. The reciprocal adsorption P₄, with EP antiserum, was less spectacular because of the lack (except WV6) of heterologous reactors. The homologous titer, however, was reduced from 40,960 to 20,480. On such a basis it

TABLE 3
ADSORPTION EXPERIMENTS WITH ANTI-P₄ SERUM

ORGANISM AGGLUTINATED	BEFORE ADSORP- TION	ADSORBED WITH					
		11	4	CA ₂	CA ₁	EP	P ₄
P ₄	20480	5120	2560	1280	1280	1280	—*
CA ₁	5120	5120	5120	—	—	—	—
CA ₂	2560	1280	1280	—	—	—	—
11.....	160	—	—	160	80	—	—
4.....	160	—	—	80	80	—	—

* —, Less than 1:40.

would be easy to conclude that EP does have factors in common with P₄, which do not manifest themselves by direct agglutination. EP likewise reduced to a considerable extent the homologous and heterologous titers in CA₁ and CA₂ sera.

NATURE OF CROSS-REACTION

Virtually all the cross-agglutination that occurred with the soft-rot organisms took place in a floccular manner. This type of reaction has been shown to be due, in other groups of bacteria, to flagellar components and their corresponding agglutinins. That this is also true in the soft-rot group can be shown by the following experiment.

It was pointed out by WHITE (22) that the flagellar components of *Salmonella* organisms were destroyed, with no impairment to the somatic fractions, by growing the organisms on phenol agar (1:1000) and then heating the growth in a water-bath at 100° C. for 30 minutes. Such suspensions are homogeneous and agglutinate only in a coarse, granular fashion, indicative of reaction between somatic components and corresponding agglutinins. The commonly used alcohol precipitation method did not give satisfactory results with the *Erwinia*.

Inasmuch as the antisera contained both flagellar and somatic agglutinins, in order to obtain a serum which affected only the flagellar components, all that was necessary was to absorb any one serum with the homologous phenol-heated antigen. After several such adsorptions, all the somatic antibodies were removed. There was, however, no loss of titer of the cross-reactions, when such an adsorbed serum was used in agglutination tests. This indicated conclusively that the cross-reactions in the group are due to common flagellar components (table 4).

TABLE 4
RESULTS OF FLAGELLAR CROSS-AGGLUTINATION EXPERIMENTS

ORGANISM AGGLUTINATED	IMMUNE SERA PREPARED AGAINST AND ADSORBED WITH HOMOLOGOUS PHENOL-HEATED ORGANISMS												
	4	EA	EMN	11	NZ	ES	496	P ₄	EP	CA ₁	CA ₂	WV ₄	WV ₆
4.....	5120	80	320	10240	—	—	—	160	—	—	—	—	—
EC.....	—*	—	—	—	—	—	—	—	—	—	—	—	—
EA.....	—	—	—	—	80	—	—	—	—	—	—	—	—
5.....	640	—	160	640	—	—	—	—	—	—	—	—	—
EMN.....	80	—	2560	160	—	—	320	—	—	—	—	—	—
11.....	320	160	320	20480	—	—	—	160	—	—	—	—	—
WV ₃	—	—	—	—	—	—	—	—	—	—	—	—	—
494.....	—	—	—	—	—	—	—	—	—	—	—	—	—
NZ.....	—	5120	—	—	10240	5120	—	—	—	—	—	—	—
495.....	—	—	—	—	—	—	—	—	—	—	—	—	—
ES.....	—	640	—	—	5120	10240	—	—	—	—	—	—	—
496.....	—	—	—	—	—	—	5120	—	—	—	—	5120	—
P ₄	640	—	—	320	—	640	—	5120	160	2560	80	—	—
EP.....	—	—	—	—	—	320	—	—	20480	—	—	5120	20480
CA ₁	—	—	—	—	—	160	—	2560	—	1280	1280	—	—
CA ₂	—	—	—	—	—	—	—	1280	—	5120	1280	—	—
WV ₄	—	—	—	—	—	—	2560	—	5120	—	—	10240	—
WV ₆	—	—	—	—	—	—	—	—	5120	—	—	—	81920

* —, less than 1:40.

Experimentation with sera prepared against phenol-heated antigens revealed the opposite of the results found with the flagellar components. With either living or phenol-heated antigens, there was little evidence of cross-agglutination (table 5). Only in cases where cross-adsorption tests revealed that the organisms were identical, or nearly so, were there cross-reactions. The agglutination tests for somatic components were incubated 18 hours at the normal temperature. The titers prepared against phenol-heated antigens were also exceedingly low. Such results are in accord with those of WHITE with *Salmonella* somatic sera.

Adsorption experiments were also conducted with respect to the somatic components. The bulk of the adsorptions were carried out with fresh packed cells, although some were done with phenol-heated organisms. Inasmuch as the flagellar components were of no consequence, the use of untreated packed cells was more

convenient. The data from such experiments are meager because of but a minimum of somatic cross-agglutination. In the case of NZ and EA, however, it was demonstrated that the components in each were the same. This was also deducible from straight adsorption experiments. In the CA₁, CA₂, and P₄ series it was shown that CA₂ had a common somatic component with CA₁, but that the latter possessed one in excess. This was the only determinable serological difference between CA₁ and CA₂, inasmuch as their flagellar components were the same.

TABLE 5
RESULTS OF SOMATIC CROSS-AGGLUTINATION EXPERIMENTS

ORGANISM AGGLUTINATED	IMMUNE SERA PREPARED AGAINST PHENOL-HEATED ORGANISMS								
	4	EA	EMN	NZ	ES	P ₄	EP	CA ₁	CA ₂
4.....	5120	—	80	—	—	—	—	—	—
EC.....	—*	—	—	—	—	—	—	—	—
EA.....	—	80	—	320	320†	—	—	—	—
5.....	640	—	—	—	—	—	—	—	—
EMN.....	320(?)	—	640	—	—	—	—	—	—
11.....	1280	—	40	—	—	—	—	—	—
WV3.....	—	—	—	—	—	—	—	—	—
494.....	—	—	—	—	—	—	—	—	—
NZ.....	—	80	—	320	160†	—	—	—	—
495.....	—	—	—	—	—	—	—	—	—
ES.....	—	—	—	80†	640	—	—	—	—
496.....	—	—	—	—	—	—	—	—	—
P ₄	—	—	—	—	—	320	—	80	—
EP.....	—	—	—	—	—	—	5120	—	—
CA ₁	—	—	—	—	—	—	—	320	160
CA ₂	—	—	—	—	—	—	—	160	320
WV4.....	—	—	—	—	—	—	—	—	—
WV6.....	—	—	—	—	—	—	—	—	—

* —, less than 1:40.

† Floccular; all others granular.

It is obvious that there were too few opportunities for experimentation with somatic components. The 4, 11, 5, and EMN group likewise showed variance in somatic factors. That such factors were not absolutely specific for each organism is to be expected. It is hardly conceivable that each type would possess an individual somatic component. It is fairly certain, however, that the somatic components are far more type specific than are those of the flagella.

Carbohydrate materials were extracted from all the organisms of the soft-rot group. In every case the material gave a strong Molisch test for the furan ring (indicative of some type of polysaccharide) and yielded a negative or weakly positive Biuret reaction. Those weakly positive could be made negative through further purification by a second acetone precipitation. The carbohydrate material was also found able to resist tryptic digestion with no impairment of its precipitative ability.

The material was highly reactive in precipitation experiments, giving strong ring tests even when diluted (in some cases) to 1:100,000. The titers differed in some respects according to the individual sera. Even with low titering agglutinating sera, a strong precipitin test was often observed. With one serum (EAO) prepared after the usual course of injections, which exhibited an agglutination titer of 1:80, a good precipitin result was obtained.

The result of the precipitin tests (table 6) paralleled in every respect the somatic agglutination. It therefore became desirable to ascertain whether it was the

TABLE 6*
PRECIPITATION CROSS-REACTIONS WITH EXTRACTED CARBOHYDRATE MATERIALS

ORGANISM PRECIPITATED	IMMUNE SERA PREPARED AGAINST											
	4	EA	EMN	NZ	ES	P ₄	EP	CA ₁	CA ₂	496	WV6	WV ₄
4.....	+	-	±	-	-	-	-	-	-	-	-	-
EC.....	-	-	-	-	-	-	-	-	-	-	-	-
EA.....	-	+	-	+	-	-	-	-	-	-	-	-
5.....	+	-	-	-	-	-	-	-	-	-	-	-
EMN.....	±	-	+	-	-	-	-	-	-	-	-	-
11.....	+	-	-	-	-	-	-	-	-	-	-	-
WV ₃	-	-	-	-	-	-	-	-	-	-	-	-
494.....	-	-	-	-	-	-	-	-	-	-	-	-
NZ.....	-	+	-	+	-	-	-	-	-	-	-	-
495.....	-	-	-	-	-	-	-	-	-	-	-	-
ES.....	-	-	-	-	+	-	-	-	-	-	-	-
496.....	-	-	-	-	-	-	-	-	-	+	-	-
P ₄	-	-	-	-	-	+	-	-	-	-	-	-
EP.....	-	-	-	-	-	-	+	-	-	-	-	-
CA ₁	-	-	-	-	-	-	-	+	±	-	-	-
CA ₂	-	-	-	-	-	-	-	±	+	-	-	-
WV ₄	-	-	-	-	-	-	-	-	-	-	-	+
WV ₆	-	-	-	-	-	-	-	-	-	-	+	-

* +, Strong reaction; ±, weak reaction; -, no reaction.

same reactive material in each case. Experiments showed this to be so. For example, extraction of material from phenol-heated cells which had been carefully washed gave results identical with those of whole cell extractions. Likewise, carbohydrate antigens precipitated strongly with sera prepared against phenol-heated antigens.

Adsorption experiments also showed the carbohydrate material to be bound up in the somatic portion of the cell. The employment of sera which had been prepared for testing flagellar agglutinations resulted in negative precipitin tests. This in spite of the fact that there still remained strong flagellar agglutinating factors.

Discussion

Although it has been apparent for a number of years that the soft-rot group of organisms is exceedingly heterogenetic from a serological standpoint (3, 10, 14,

15, 16, 19, 21), it should not be considered improbable that definite serological groups might be encountered. It is apparent from this report that such a division as maltose fermentation might well divide the soft-rot group into two quite compact serological groups. It is realized, however, that the number of cultures used in these experiments was small. Nevertheless, the fact remains that there is a high percentage of cross-agglutination within the groups. This percentage is far higher than might possibly occur by chance, as can be proved by applying statistical methods. It is the contention of the writer that the comparatively high percentage of cross-reaction found within the maltose+ and maltose- groups, together with the resulting high titers, is indicative of considerable serological homogeneity. Experimentation carried on at the present time has revealed many common antigenic components. This is especially true within the two fermentative groups, although there is evidence of common antigenic factors among maltose+ and maltose- groups.

It is possible also that the high degree of serological heterogeneity is due to the presence of numerous inagglutinable strains. If these organisms were used for adsorption, however, their relationship would become evident and a far higher degree of serological homogeneity would result. Hence it is apparent that EP belongs in the CA1, CA2, P4 group, although it fails to agglutinate in any of the corresponding sera. A similar situation has been recorded in the *Salmonella* by WHITE (23).

The present experiments have shown that the flagellar components of the soft-rot organisms are group specific in nature, whereas the somatic elements are apparently type specific. It has been pointed out by others that the flagellar components of the genus *Salmonella* are type specific in nature and the somatic fractions group specific. In the genus *Proteus*, however, this situation is reversed: the common antigenic fractions are flagellar and the somatic elements are type specific. It is evident then that my group is analogous to the *Proteus* in its antigenic makeup. This is further emphasized on comparing the action of extracted carbohydrate materials. FURTH and LANDSTEINER (7) have demonstrated that, in the *Salmonella*, carbohydrate materials are derived from the somatic portion of the cell and immunologically act in the group-specific manner as do the somatic antigens. ELROD (6) has found this to be true of *E. amylovora*.

PRZESMYCKI (18) has shown the situation to be different in the genus *Proteus*. Here extracted carbohydrate materials are specific, even as the somatic elements. It may be that the serological heterogeneity found in the *Erwinia* and the *Proteus* groups is in some way linked with the flagellar components being group specific.

Summary and conclusions

1. Eighteen cultures of *Erwinia carotovora* (soft-rot group) were tested for fermentative ability in twenty-six substances. Division on the basis of maltose fer-

mentation found ten fermenting the sugar with the formation of either acid or acid and gas, while eight failed to ferment the carbohydrate. The maltose— organisms were agglutinated in 33.3 per cent of the cases with maltose— antisera. There was 28.6 per cent of cross-reaction with the maltose+ cultures and maltose+ antisera. Between the two fermentative groups there was but 7.5 per cent of cross-reaction. The total number of cross-agglutination experiments produced with thirteen antisera was 18.1 per cent, the majority of which occurred within the two fermentative groups.

2. A division on the basis of sorbitol fermentation may be significant and is considered worthy of further investigation.

3. The correlation between maltose fermentation and serological groups seems to bear out the contention of others that there are two soft-rot pathogens. These two groups, however, show within them considerable variation in fermentative powers and antigenic structure.

4. The common antigenic components of the group are bound up in the flagella. Adsorption experiments showed a large number of flagellar components.

5. The somatic fractions were shown to be type specific. There was, however, some evidence of common somatic factors.

6. Carbohydrate materials extracted from the organisms proved to be specific, and paralleled the somatic results. In all probability the somatic fractions owe their specificity to these carbohydrate materials.

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LITERATURE CITED

1. BERGEY *et al.*, Manual of determinative bacteriology. 5th ed. Baltimore 1939.
2. BONDE, R., Comparative studies of bacteria associated with potato blackleg and seed-piece decay. *Phytopath.* 29:831-851. 1939.
3. BROOKS, R. ST. J., NAIN, K., and RHODES, MABEL, The investigation of phytopathogenic bacteria by serological and biochemical methods. *Jour. Path. and Bact.* 28:203-209. 1925.
4. DOWSON, W. J., On the systematic position and generic names of the Gram-negative bacterial plant pathogens. *Zentralblatt Bakt. Parasitenk. Abt. II.* 100:177-193. 1939.
5. ———, Identity of the bacteria causing potato blackleg. *Nature* 145:263. 1940.
6. ELROD, R. P., Serological studies of the Erwinae. I. *Erwinia amylovora*. *BOT. GAZ.* 103: 123-131. 1941.
7. FURTH, J., and LANDSTEINER, K., Studies on the precipitable substances of bacilli of the *Salmonella* group. *Jour. Exp. Med.* 49:727-743. 1929.
8. HARDING, H. A., and MORSE, W. J., The bacterial soft-rot of certain vegetables. I. The mutual relationships of the causal organisms. *N.Y. Agr. Exp. Sta. Tech. Bull.* 11. 1909.
9. JENNISON, H. M., Potato blackleg with reference to the etiological agent. *Ann. Mo. Bot Gard.* 10:1-72. 1923.

10. LACEY, MARGARET S., A soft-rot of potato tuber due to *Bacillus carotovorus* and a comparison of the cultural, pathogenic, and serological behavior of various organisms causing soft-rots. Ann. Appl. Biol. 13:1-11. 1926.
11. LEACH, J. G., Blackleg diseases of potatoes in Minnesota. Univ. Minn. Agr. Exp. Sta. Bull. 76. 1931.
12. LINK, G. K. K., and TALIAFERRO, W. H., Further agglutination tests with bacterial plant pathogens. II. Soft-rot group: *Bacillus aroideae* and *Bacillus carotovorus*. BOT. GAZ. 85: 198-207. 1928.
13. MASSEY, A. B., A study of *Bacillus aroideae* Townsend, the cause of soft-rot of tomato, and *Bacillus carotovorus* Jones. Phytopath. 14:460-477. 1924.
14. MATSUMOTO, T., Studies on some phytopathogenic bacteria with special reference to agglutination and complement fixation. Jour. Soc. Trop. Agr. 1:155-171. 1929.
15. MATSUMOTO, T., and OKABE, N., On the causal organisms of bacterial soft-rots of Kotvoran, *Pholaenopsis aphrodite*. Jour. Soc. Trop. Agr. 3:117-134. 1931.
16. MATSUMOTO, T., and SAMAZOWA, K., On the relationship between serological reaction and other biological characters of some putrefactive phytopathogenic bacteria. Jour. Soc. Trop. Agr. 3:317-336. 1931.
17. MORSE, W. J., Studies on the blackleg disease of the potato with special reference to the relationship of the causal organisms. Jour. Agr. Res. 8:79-126. 1917.
18. PRZESMYCKI, F., Analyse des éléments antigènes des souches du *Proteus* HX₁₉, et X₁₉O. Compt. rend. Soc. biol. 95:744. 1926.
19. STANLEY, A. R., Physiologic and serologic studies of the soft-rot and colon group of bacteria. W.Va. Agr. Exp. Sta. Bull. 287. 1938.
20. STANLEY, A. R., and ORTON, C. R., Bacterial stalk-rot of sweet corn. (Abst.) Phytopath. 22:26. 1932.
21. STAPP, C., Die Schwarzbeinigkeit und Knollennassfaule der Kartoffel. Arb. aus der biolog. Reichsanstalt f. Land. und Forstwirtschaft. 16:643-703. 1928.
22. WHITE, P. B., Further studies of the *Salmonella* group. Med. Res. Coun. Rep. 103. 1926.
23. WHITE, P. B., in SAVAGE, W. G., and WHITE, P. B., An investigation of the *Salmonella* group, with special reference to food poisoning. Med. Res. Coun. Rep. 91. 1925.

EFFECT OF BORON ON GROWTH AND DEVELOPMENT OF THE RADISH

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 531

JOHN SKOK

(WITH FIVE FIGURES)

Introduction

AGULHON (1) found that different species of plants reacted differently toward boron, and that for each there was an optimum concentration which favored growth and yield of the plant, although he did not describe the deficiency symptoms in detail. Later HASELHOFF (14) reported that boron increased growth in beans; BRENCHLEY (3) found peas to grow better with boric acid; and MAZÉ (20) found boron was required by maize and added it to his list of essential elements. WARINGTON (29) and SOMMER and LIPMAN (28) showed definitely that boron was essential for the normal growth of the broad bean and a number of other plants. Several excellent summaries are available (4, 12, 15, 22, 33) of more recent work on this subject.

The exact role of boron is still not known. It has been possible merely to speculate as to its function by noting the development of plants when it is absent. Much has been learned from work done during the past 20 years concerning gross deficiency symptoms and histological and metabolic effects. PURVIS (24) has reviewed the effects of boron and the factors affecting its deficiency in the soil. In general, boron-deficient plants show disintegration of the meristematic and conducting tissues and the eventual death of first the growing tip and finally the entire plant, retarded root development, and brittleness of the leaf blades and petioles. EATON (8) found that the effects of boron deficiency are first noticeable in the youngest and of boron excess in the oldest portions of the plant. Some investigators (7, 21) think boron functions as a hormone or enzyme or is essential to the formation of substances which are activators or regulators of metabolic processes, while others (6, 18, 29) question the hormonal or enzymatic role. There are some indications that boron may have an effect on calcium absorption (6, 32) and utilization (19, 27); and microchemical studies by SHIVE (26) suggest that it is intimately involved in protein metabolism. Both ammonium nitrogen and sugar accumulate in minus boron plants. This strongly suggests that boron deficiency interferes with the aminization of the carbohydrate derivatives and in this way interferes with metabolic activity in meristematic regions.

In the present experiments a study was made of the effect of boron on the

growth and development of the radish. AGULHON (1) included the radish in his studies and found a definite increase in growth with boron, but he did not describe the deficiency symptoms. SHIVE (26) found definite deficiency symptoms in the Long Icicle variety 6 days after germination. The first foliage leaves became discolored and distorted, and finally the growing tips died. He did not describe symptoms of the hypocotyl or root. PURVIS and HANNA (25) found that among a number of other plants the radish showed boron deficiency symptoms in various fields in Virginia. They state that the symptoms consisted of root malformations and an internal darkening very similar to that found in turnips.

Material and methods

Seeds of *Raphanus sativus* L., variety Scarlet Globe, were planted in flats of clean quartz sand, and after 10–12 days plants of uniform size were transplanted to glazed earthenware pots filled with the same material. All plants received a minus boron nutrient until after transplanting and then received applications of the appropriate nutrient solutions every second day. The solutions were made up with distilled water and Merck's reagent quality chemicals of the following concentrations: 0.006M $\text{Ca}(\text{NO}_3)_2$; 0.0045M KH_2PO_4 ; 0.0045M MgSO_4 ; 0.5 p.p.m. Fe added as ferric citrate; 0.5 p.p.m. Mn added as MnCl_2 ; 0.5 p.p.m. Zn added as ZnCl_2 ; and 0.125 p.p.m. Cu added as CuCl_2 . The plus boron solution received in addition 0.5 p.p.m. B added as H_3BO_3 . The plants were harvested 39–42 days after the seeds were planted. Fresh and dry weights of the top and root portions and volumes of the root portions were taken. The volumes were determined by the water displacement method. The term root as used here includes the enlarged underground portion which consists of the hypocotyl as well as the root. Percentage dry weights and top-root ratios were also determined.

Experimentation

These experiments were of three types: (A) a study of the gross boron deficiency symptoms of the radish, (B) a study of the effect of photoperiod on the manifestation of the deficiency symptoms, and (C) a histological study.

A. GROSS BORON-DEFICIENCY SYMPTOMS

Three series of plants were grown under different treatments. The plants of series 1 received boron throughout the period of the experiment. The seeds were planted April 6, 1939, and the plants were harvested 41 days later. The plants of series 2 received boron for 18 days after planting, at which time the plants were 3–4 cm. tall and had four leaves. From then on they received a minus boron nutrient. They were planted and harvested at the same time as the plants in series 1. The plants of series 3 were grown without boron throughout the experi-

ment. They were planted May 12, 1939, and harvested 39 days later (table 1, fig. 1).

The plus boron plants of series 1 had large green leaves 15-20 cm. long and 5-6 cm. broad. The thickened portions were large, bright red, solid, and ranged from 2 to 4 cm. in diameter.

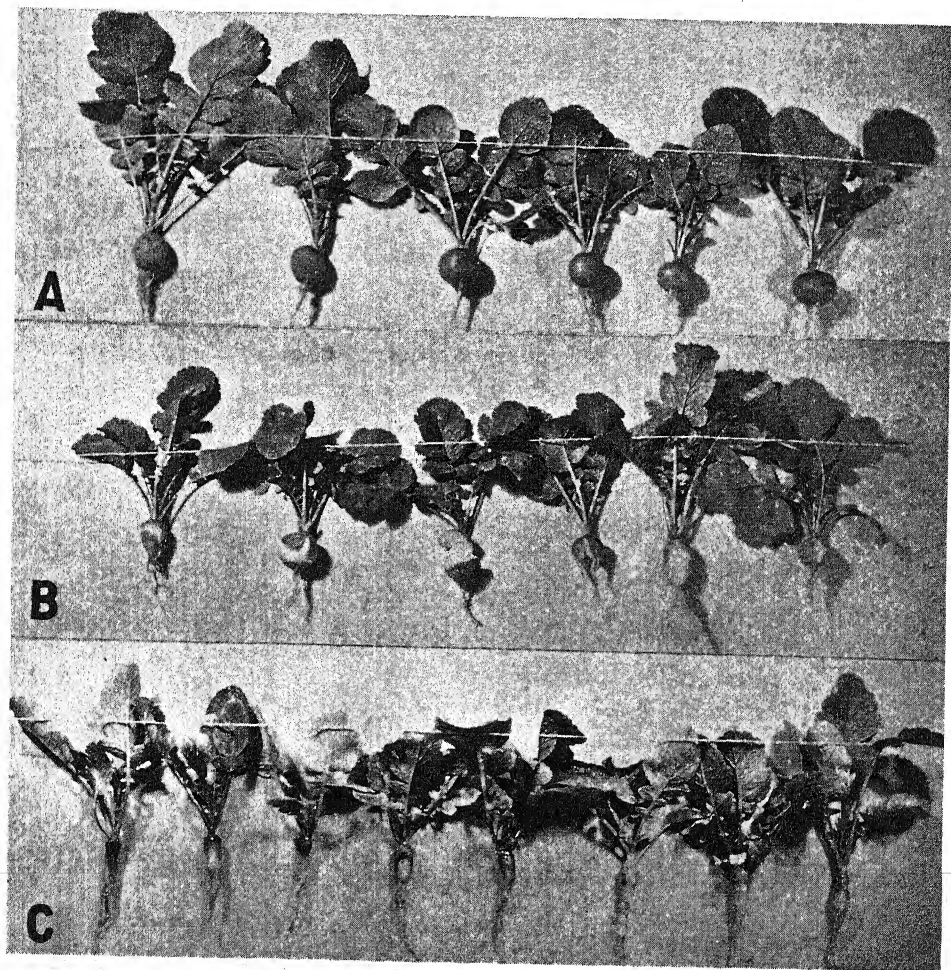


FIG. 1.—A, series 1, plus boron plants; B, series 2, plus boron for first 18 days only; C, series 3, minus boron plants.

The top portions of the plants in series 2, which received boron for the first 18 days, were well developed and similar to those in the first series, although the leaves were slightly lighter and some were distinctly mottled. The plants were also slightly smaller and the petioles were rather brittle as compared with those of the plus boron plants. The percentage dry weight was nearly the same. The

enlarged portions of this series were all light in color, and all of them were much cracked. The splitting was usually longitudinal but in some cases was also transverse. The cracks in some cases were shallow and in others were very deep; a few radishes were split almost in half. The size of the thickened portion was slightly smaller than that of the plus boron series, as is shown in table 1.

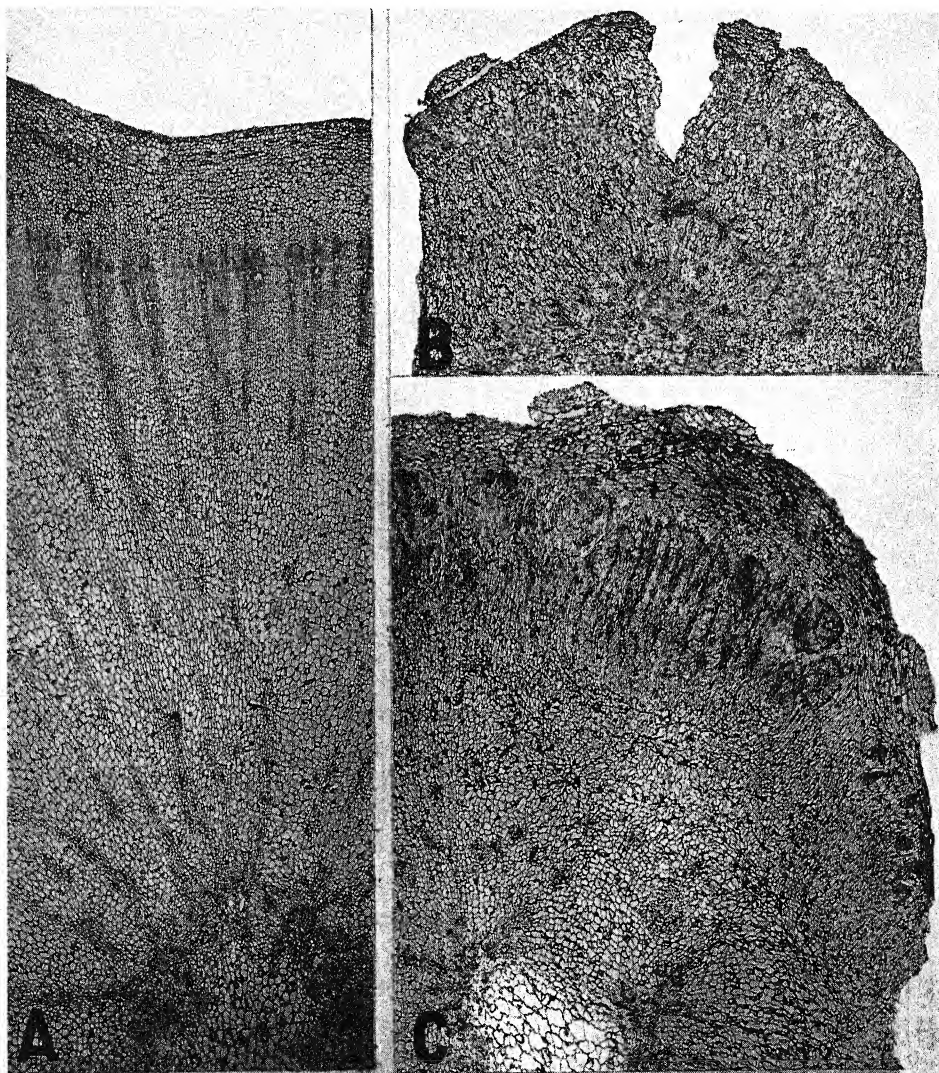


FIG. 2.—Section through hypocotyl region: *A*, plus boron; *B*, *C*, minus boron. *B* and *C* show unaffected vascular tissue at central portion of axis and absence of lignified vessels as well as of disintegrated phloem in area between central vascular tissue and cambium region. Split section (*B*) shows indistinctly developed vascular bundles, and unsplit section (*C*) shows dome arrangement of bundles at cambium region.

The plants of series 3, which were continuously on a minus boron nutrient, were much smaller than either of the other groups. The leaves were 8-15 cm. long, very pale, some extremely chlorotic, the petioles brittle and easily broken when handled. The chlorosis was confined to the areas between the veins, the veins themselves being usually green. Some of the older leaves were curled downward, but generally they were not markedly deformed. The young leaves in the center were very small, curled, thick, and usually brittle. The total growth of the tops as measured by dry weight was not much less than that in the plus boron series, but the total root growth was only about half as great. The percentage dry weight was also greater in this group than in either of the other two. The hypocotyl and

TABLE 1

DATA OF EXPERIMENTS OF PART A. VOLUMES AND WEIGHTS REPRESENT AVERAGES PER PLANT ON 40-PLANT BASIS

CONDITION	VOLUME OF ROOT (ROOT AND HYPOCOTYL) (CC.)	WET WEIGHT		DRY WEIGHT		PERCENTAGE DRY WEIGHT			WET TOP-ROOT RATIO	DRY TOP-ROOT RATIO
		TOP (GM.)	ROOT (GM.)	TOP (GM.)	ROOT (GM.)	ENTIRE PLANT	TOPS	ROOTS		
Series 1 (plus boron)...	10.19	6.27	10.19	0.64	0.63	7.75	10.22	6.22	0.62	1.01
Series 2 (plus boron for first 18 days)	8.92	5.36	8.71	0.61	0.54	8.17	11.38	6.20	0.61	1.13
Series 3 (minus boron).	4.19	5.32	4.09	0.57	0.30	9.25	10.71	7.33	1.30	1.90

root portions of the plants in this series were much elongated, narrow, and all were nearly white in color, and split. The splitting here also was usually parallel with the axis but in some cases at right angles to it. The volumes of the thickened portions of the plants in this series were less than half of those of the plus boron series and of an entirely different shape. The volumes of the thickened portions in series 2 were only slightly smaller than those of the plus boron series, and aside from the splitting, were of about the same shape.

A common boron-deficiency symptom is death of the growing tip and cessation of further top growth. The plants in these experiments showed only moderate symptoms in the tops but marked symptoms in the underground portions. SHIVE (26) reported that lack of boron resulted in complete destruction of the growing tip in radishes grown in sand which was treated with acid. The sand used in these experiments was not so treated and may have contained minute traces of boron. The boron-deficient radishes reported by PURVIS and HANNA (25) showed symp-

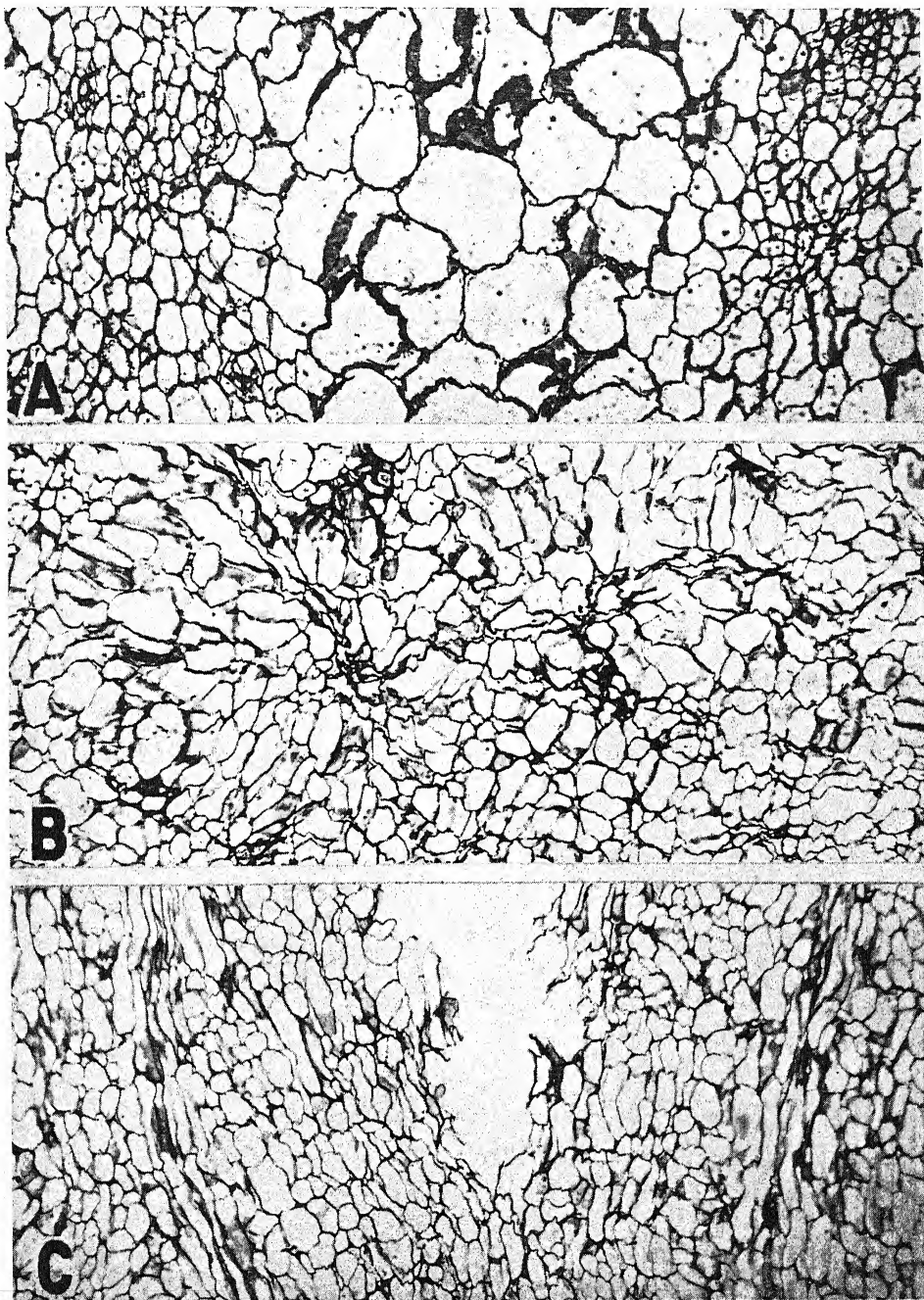


FIG. 3.—Section through hypocotyl in area between central portion of axis and cambium region: *A*, plus boron, showing vascular bundles and large xylem parenchyma cells; *B*, *C*, minus boron, showing absence of lignified vessels, disintegrated phloem cells, and smaller size of xylem parenchyma cells.

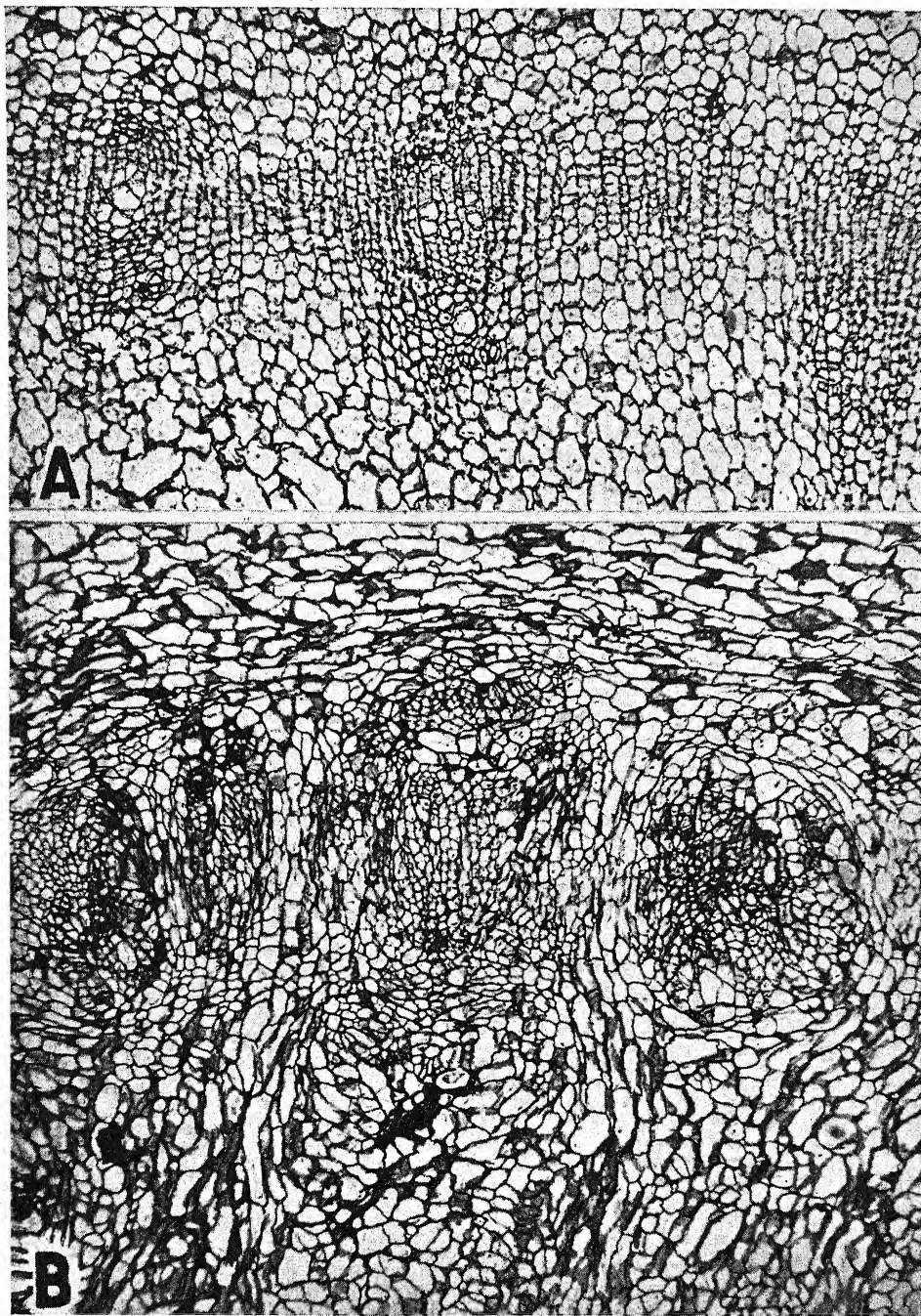


FIG. 4.—Cambium region: *A*, plus boron; *B*, unsplit portion of axis of minus boron plant showing dome arrangement of bundles and proliferation of xylem parenchyma and ray cells.

toms somewhat similar to those found in these experiments (root malformation), but they did not show any splitting. Their plants were grown in a boron-deficient soil, and possibly the deficiency was not so nearly complete. The plants of series 2, which received boron for the first 18 days, showed definite symptoms although less severe.

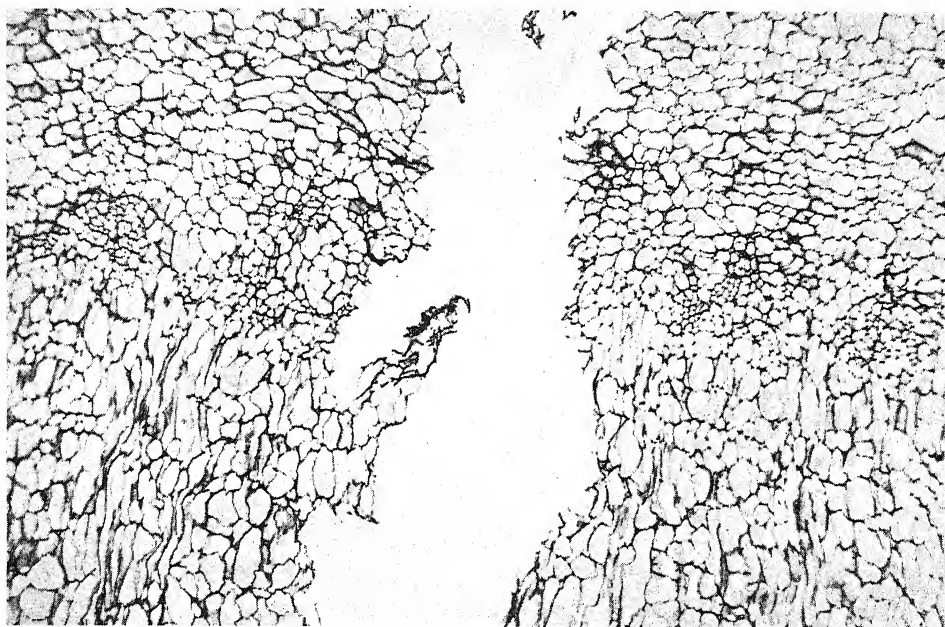


FIG. 5.—Cambium region of split portion of axis of minus boron plant showing indistinct vascular bundles, slightly lignified vessels, disintegrated phloem and cambium cells, and some proliferation of xylem parenchyma and ray cells.

B. EFFECT OF PHOTOPERIOD ON BORON-DEFICIENCY SYMPTOMS

Two experiments were conducted. In experiment 1 the seeds were planted May 17, 1940, and the plants harvested 42 days later. They were grown under plus and minus boron conditions; the minus boron plants received no boron throughout the experiment. One hundred and thirty plants were grown under each condition. Both boron series were grown under a short photoperiod of 7 hours (9:00 A.M. to 4:00 P.M.). Experiment 2 in this study was conducted as a check on the results of experiment 1 and on the boron-deficiency symptoms obtained in the former group of experiments, which were conducted under long photoperiod. In experiment 2 the plants were grown under the following four conditions: plus boron, long photoperiod; plus boron, short photoperiod; minus

boron, long photoperiod; minus boron, short photoperiod. The short photoperiod was also 7 hours, and the long photoperiod was the natural day length of that time (about 15 hours). The plants of experiment 2 were planted July 16, 1940, and were harvested 41 days later. Seventy plants were grown under each condition.

EXPERIMENT 1.—Under short photoperiod the boron-deficiency symptoms were not so pronounced as compared with the plus boron plants. The minus boron plants were slightly smaller and somewhat lighter in color. Their petioles were rather brittle and easily broken. The root-hypocotyl portions were smaller, more elongated, and a little lighter in color. Splitting occurred in only a few instances and in no case was so great as in the plants of the former experiments. Out of the total number of minus boron plants in this experiment, approximately 73 per cent were not split, 15 per cent had very slight splitting, and 12 per cent had more pronounced splitting (table 2).

TABLE 2
DATA OF EXPERIMENT 1, PART B. VOLUMES AND WEIGHTS REPRESENT
AVERAGES PER PLANT ON 130-PLANT BASIS

GROWN UNDER 7-HOUR PHOTOPERIOD	VOLUME OF ROOT (ROOT AND HYPO- COTYL) (CC.)	WET WEIGHT		DRY WEIGHT		PERCENTAGE DRY WEIGHT			WET TOP- ROOT RATIO	DRY TOP- ROOT RATIO
		TOP (GM.)	ROOT (GM.)	TOP (GM.)	ROOT (GM.)	ENTIRE PLANT	TOPS	ROOTS		
Plus boron.....	4.61	7.24	4.56	0.52	0.25	6.51	7.20	5.40	1.59	2.12
Minus boron....	3.31	6.20	3.28	0.47	0.18	6.90	7.57	5.63	1.89	2.54

EXPERIMENT 2.—The plus and minus boron plants grown under long photoperiod responded similarly to those in series 1 and 3 in the study of gross boron-deficiency symptoms. Thirty days after the time of planting, the tops of the long photoperiod, minus boron plants were smaller and had narrower leaves than those receiving boron. The leaves had a bluish green color, very brittle petioles, and were rather stiff and curled. At this time there was scarcely any difference between the appearance of the top portions of the plus and minus plants grown under short photoperiod. The petioles of the minus boron plants, however, were somewhat stiffer. At the time of harvesting, the long-photoperiod, plus boron plants had well-developed large green leaves and underground portions 3.5-4 cm. in diameter and bright red in color. The leaves of the minus boron plants were smaller, bluish green in color, curled, and brittle. The young leaves were deformed and also brittle. The underground portions were light in color, long, and very slender, and all were cracked. They were 5-8 cm. long and 0.5-2 cm. in diameter. The leaves of the short-photoperiod, plus boron plants were lighter in color and

smaller than those of the long-photoperiod group. The underground portions were also considerably smaller, but well formed and bright red in color. The leaves of the short-photoperiod, minus boron plants were of about the same size and color as those of the plus boron plants, but they were also brittle. The underground portions varied greatly in size, but their average was not much below that of the plus boron group. They were lighter in color and somewhat deformed and had about the same percentage of cracking as similar plants of experiment 1. The data of table 3 show that on weight and volume bases the plus and minus boron plants grown under short photoperiod made about the same amount of growth, but both groups made less growth than the minus boron plants grown under long

TABLE 3
DATA OF EXPERIMENT 2, PART B. VOLUMES AND WEIGHTS
REPRESENT AVERAGES PER PLANT ON 70-PLANT BASIS

CONDITION	VOLUME OF ROOT (ROOT AND HYPOCOTYL (CC.))	WET WEIGHT		DRY WEIGHT		PERCENTAGE DRY WEIGHT			WET TOP-ROOT RATIO	DRY TOP-ROOT RATIO
		TOP (GM.)	ROOT (GM.)	TOP (GM.)	ROOT (GM.)	ENTIRE PLANT	TOPS	ROOTS		
Plus boron, long photoperiod...	12.37	19.28	11.68	1.14	0.55	5.43	5.90	4.67	1.65	2.09
Minus boron, long photoperiod...	4.67	10.58	4.61	0.77	0.29	6.96	7.27	6.23	2.30	2.68
Plus boron, short photoperiod...	3.55	9.75	3.52	0.62	0.18	6.00	6.33	5.07	2.77	3.46
Minus boron, short photoperiod.....	3.06	9.99	3.07	0.64	0.17	6.21	6.36	5.70	3.26	3.63

photoperiod. The long-photoperiod, minus boron plants exhibited the most severe deficiency symptoms. In terms of appearance and general condition, it is evident that the plus boron plants make relatively much greater growth under long photoperiod and the minus boron plants make more growth under short photoperiod. This is probably a matter of differential rate of growth, dependent on the photoperiod, with subsequent differential nutrient requirement. The long-photoperiod plants grow more vigorously and synthesize more materials and require more nutritive substances in order to do so. If an essential element is lacking, therefore, the deficiency symptoms in such plants are more severe than in less vigorously growing plants such as those in the short-photoperiod group. This is in agreement with EATON'S (8) finding that the more vigorously growing plants exhibit more pronounced deficiency symptoms, and with those of WARINGTON (31) that the boron-deficiency symptoms of several plants were less pronounced under short than under long photoperiod.

Although the radish ordinarily flowers under long photoperiod (10, 11), only a few plants out of the entire numbers grown did so. At the same time other plants growing in the garden bloomed. This difference in the flowering response may be caused by difference in temperature or in light intensity. PLITT (23) found photo-thermal responses of the radish.

C. HISTOLOGICAL EFFECTS OF BORON DEFICIENCY

The root-hypocotyl portions of plus and minus boron plants were used in the histological study. The material was preserved in alcohol-acetic acid-formalin solution, dehydrated in an alcohol-tertiary butyl-alcohol series, imbedded, and cut at 7 μ . Samples were taken at 20 days after planting and every 4 days thereafter, until time of harvesting. Any differences shown in the plus and minus boron material in the early samples were more pronounced in later stages, and for that reason photomicrographs were taken only of material collected at the time of harvesting. Slides were made of material from two different experiments (plants grown a year apart), and the histological effect of boron deficiency was found to be similar in each case. The materials for the histological study were taken from plants grown under natural long photoperiod (about 15 hours).

A number of reports have shown that boron is important in the differentiation of the vascular system, and that in its absence there is general disintegration of the cambium and phloem. AGULHON (1) noted that boron is especially abundant in lignified tissue and bark, which indicated that it may have some function in the formation of vascular tissue or the compounds which impregnate these tissues. WARINGTON (30) found that boron deficiency in *Vicia faba* caused abnormal structure of both the stem and root, the chief features being: (a) hypertrophy of the cells of the cambium followed by degeneration with discoloration, or direct disintegration of the same tissue without previous enlargement; (b) frequent disintegration of phloem and ground parenchyma; and (c) slight development of xylem and in some cases ultimate disintegration of this tissue. BRENCHLEY and THORNTON (5) found that the vascular bundles related to the nodules on the roots of *Vicia faba* either failed to develop entirely or only to a small extent when boron was lacking. Anatomical studies on the tomato by JOHNSTON and DORE (16, 17) and by FISHER (9) showed disintegration of the phloem of the petioles and stems of boron-deficient plants. The total sugars and starch were more abundant in the leaves of the minus boron than in the plus boron plants. This was thought to be caused by injury to the conductive tissues, which reduced their capacity to translocate sugars. HAAS and KLOTZ (13) found boron to be essential for cell division in meristematic tissue of growing points such as buds, and equally essential for cambial activity. They found that the cambium and phloem disintegrated in minus boron citrus plants. The xylem was affected to a less extent. They also

found that sugar accumulated in the leaves of the affected plants because of the disintegrated conducting system, and that upon the addition of boron to these cultures there was rapid growth of new conductive tissue followed by decrease in the sugar content of the leaves.

CHANDLER (2) studied boron deficiency in eleven species of *Brassica*. In boron-deficient broccoli and Brussels sprouts he found that cell division stopped in the root tips and the root cap disintegrated. In rutabaga he observed crushing of meristematic cells of the stem tip, and in the root he found crushing of cortical cells, accumulation of cell inclusions in xylem parenchyma cells, elongation of certain cells near the cambium, and failure of the cork cambium to form cork. He also found crushing of cells in the cortex of the stem of cabbage, and when leaf blades of these boron-deficient plants were cut a cork cambium did not develop, but with similar treatment to plants supplied with boron, a cork cambium developed and healing occurred.

The effects of boron deficiency on the structure of the radish are somewhat similar to those in the preceding plants in that the vascular tissues are affected rather markedly. Sections through the hypocotyl of plus and minus boron plants show the general structure and the tissues affected by the deficiency (fig. 2). The vascular tissue near the center of the axis, including the primary tissue, was not affected. This is the first differentiated vascular tissue, and small amounts of boron may have been present from the seed or as impurities in the chemicals and sand, at the time of its maturation. After this amount of boron is exhausted the vascular tissue developing later would be lacking an element necessary for its development. Injurious effects on this later-forming vascular tissue again indicate that boron cannot be reutilized but that a continuous supply is necessary.

The vascular tissues occupying the area between the central, first formed, uninjured vascular tissue and the outer cambium region were markedly affected (fig. 3). Normally developed and lignified vessels in this area were entirely absent. The phloem cells disintegrated and left dead, dark-staining areas. The xylem parenchyma cells appeared to be uninjured and in some cases even rather active. The size of the xylem parenchyma cells of the boron-deficient radishes was generally smaller than those of the plus boron plants. Actual disintegration of these cells, as in the case of the ground parenchyma of *Vicia faba* reported by WARINGTON (30), was not observed.

The cambium region is of particular interest in that it shows a dual response (fig. 2). Axes which were cracked had very slightly developed vascular bundles in the cambium region near the cracks (fig. 5). Some disintegration of phloem also took place but not to the same extent as in the deeper areas. Well-developed vessels were almost entirely lacking, but some small, thin-walled vessels were

present. The cambium cells were almost entirely disintegrated. The xylem parenchyma, and particularly the ray cells, were rather active and showed proliferation.

The cambium region of axes which were not deeply cracked had an entirely different structure (fig. 4). The vascular bundles were fairly well developed and were arranged in pronounced domes and circular areas. A few vessels were present in these bundles, but they were rather thin walled, smaller, and much less lignified as compared with those of the plus boron sections. There was no pronounced phloem or cambium disintegration in these bundles, but the tissues were rather poorly developed and indistinct. Again the xylem parenchyma and particularly the ray cells were highly active. In some cases the proliferated ray cells formed complete rings around the bundles. Both these responses of the cambium region may be seen in the same axis, in which case the former is present at or near a crack and the latter where no splitting has taken place (fig. 2C).

These histological observations suggest that the splitting of the radish is a result of the failure of normal vascular tissue development, coupled with differential growth pressure. The lack of lignified xylem development and the areas of dead phloem create weak regions where the splitting may occur. The cessation of cambial growth, together with active xylem parenchyma and ray cells inside the cambium, result in internal pressure because of this unequal growth.

Summary

1. Radish plants deficient in boron produced small leaves with light bluish green color, which were (especially the petioles) brittle and curled down. The thickened underground portions were light in color, narrow and elongated, and severely cracked.

2. When boron was added up to 18 days after planting and then withheld, the top portion made greater growth but the brittleness of the leaves and the light color of the foliage were still apparent. The enlarged underground portion attained greater size and more normal shape but was also cracked.

3. Under short photoperiod the boron-deficiency symptoms were much less pronounced. Splitting of the underground portion was present in only about 27 per cent of the minus boron plants.

4. The vascular tissue near the center of the axis was not affected by boron deficiency.

5. Normally developed and lignified vessels in the area between the central, unaffected vascular tissue and the outer cambium region were entirely absent, and the phloem cells in this region had disintegrated.

6. The cambium region, if located at or near a crack, had slightly developed vascular bundles. The cambium and phloem cells were mostly disintegrated, and

well-developed vessels were lacking. The xylem parenchyma and ray cells were rather active.

7. The cambium region, if not located near a crack, had well-developed vascular bundles arranged in pronounced domes and circular areas. The vessels were unligified, but there was no pronounced phloem or cambium disintegration. The xylem parenchyma and ray cells were here also highly active.

8. The splitting is caused by the failure of normal vascular tissue development, coupled with differential growth pressure.

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LITERATURE CITED

1. AGULHON, HENRI, Recherches sur la présence et le rôle du bore chez les végétaux. Thèse, Paris. 1910.
2. CHANDLER, F. B., Mineral nutrition of the genus *Brassica* with particular reference to boron. Maine Agr. Exp. Sta. Bull. 404. 1941.
3. BRENCLEY, WINIFRED E., On the action of certain compounds of zinc, arsenic, and boron on the growth of plants. Ann. Bot. 28:283-301. 1914.
4. ———, The essential nature of certain minor elements for plant nutrition. Bot. Rev. 2: 173-196. 1936.
5. BRENCLEY, WINIFRED, and THORNTON, H. G., The relation between the development, structure, and functioning of the nodules on *Vicia faba*, as influenced by the presence or absence of boron in the nutrient medium. Proc. Roy. Soc. London B. 98:373-398. 1925.
6. BRENCLEY, WINIFRED, and WARINGTON, KATHERINE, The role of boron in the growth of plants. Ann. Bot. 41:167-188. 1927.
7. EATON, F. M., Interrelations in the effects of boron and indoleacetic acid on plant growth. Bot. Gaz. 101:700-705. 1940.
8. EATON, S. V., Effects of boron deficiency and excess on plants. Plant Physiol. 15:95-107. 1940.
9. FISHER, P. L., Responses of the tomato in solution cultures with deficiencies and excesses of certain essential elements. Maryland Agr. Exp. Sta. Bull. 375:283-298. 1935.
10. GARNER, W. W., and ALLARD, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. 18:553-607. 1920.
11. ———, Further studies in photoperiodism: The response of the plant to relative length of day and night. Jour. Agr. Res. 23:871-921. 1923.
12. GREENHILL, A. W., Boron deficiency in horticultural crops: Recent developments. Sci. Hort. 6:191-198. 1938.
13. HAAS, A. R. C., and KLOTZ, L. J., Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia 5:175-197. 1931.

14. HASELHOFF, E., Über die Einwirkung von Borverbindungen auf das Pflanzenwachstum. Landw. Versuchs-Stat. 79-80:399-429. 1913.
15. JACKS, G. V., and SCHERBATOFF, H., Soil deficiencies and plant diseases. Imperial Bureau Soil Sci., Tech. Comm. 31. 1934.
16. JOHNSTON, E. S., and DORE, W. H., The relation of boron to the growth of the tomato plant. Science n.s. 67:324-325. 1928.
17. ———, The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. 4:31-62. 1929.
18. JOHNSTON, E. S., and FISHER, P. L., The essential nature of boron to the growth and fruiting of the tomato. Plant Physiol. 5:387-392. 1930.
19. MARSH, R. P., and SHIVE, J. W., Boron as a factor in the calcium metabolism of the corn plant. Soil Sci. 51:141-151. 1941.
20. MAZÉ, P., Détermination des éléments minéraux rares nécessaires au développement du maïs. Compt. Rend. Acad. Sci. Paris 160:211-214. 1915.
21. McLEAN, R. C., and HUGHES, W. L., The quantitative distribution of boron in *Vicia faba* and *Gossypium herbaceum*. Ann. Appl. Biol. 23:231-244. 1936.
22. McMURTREY, J. E., JR., Distinctive plant symptoms caused by any one of the chemical elements essential for normal development. Bot. Rev. 4:183-203. 1938.
23. PLITT, THORA M., Some photoperiodic and temperature responses of the radish. Plant Physiol. 7:337-339. 1932.
24. PURVIS, E. R., The present status of boron in American agriculture. Soil Sci. Soc. Amer. Proc. 4:316-321. 1939.
25. PURVIS, E. R., and HANNA, W. J., Vegetable crops affected by boron deficiency in eastern Virginia. Virginia Truck Exp. Sta. Bull. 105. 1940.
26. SHIVE, J. W., The adequacy of the boron and manganese content of natural nitrate of soda to support plant growth in sand culture. New Jersey Agr. Exp. Sta. Bull. 603. 1936.
27. ———, Significant rôles of trace elements in the nutrition of plants. Plant Physiol. 16:434-445. 1941.
28. SOMMER, A. L., and LIPMAN, C. B., Evidence on the indispensable nature of zinc and boron for higher green plants. Plant Physiol. 11:321-349. 1926.
29. WARINGTON, KATHERINE, The effect of boric acid and borax on the broad bean and certain other plants. Ann. Bot. 37:629-673. 1923.
30. ———, The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. 40:27-42. 1926.
31. ———, The influence of length of day on the response of plants to boron. Ann. Bot. 47:429-458. 1933.
32. ———, Studies in the absorption of calcium from nutrient solutions with special reference to the presence or absence of boron. Ann. Bot. 48:743-776. 1934.
33. WILLIS, L. G., Bibliography of references to the literature on the minor elements and their relation to plant and animal nutrition. 3d ed. Chilean Nitrate Education Bureau, New York. 1939.

WOOD STRUCTURE OF THUJA OCCIDENTALIS

M. W. BANNAN

(WITH FORTY-THREE FIGURES)

Introduction

Because of difficulties in obtaining sufficient amounts and variety of material, anatomical studies of coniferous woods have in many instances failed to provide adequate information for the positive identification of similar woods. Before criteria can be regarded as reliable, their constancy or range of variability within the species must be determined, an undertaking which—as BAILEY and FAULL (1) have pointed out—requires the examination of much material. In the present paper the results of an extensive study of the secondary xylem of the native white cedar, *Thuja occidentalis* L., are described.

All material was collected in southern Ontario within a 75-mile radius of Toronto. Some 230 wood samples were obtained from different parts of the tree, from trees of different sizes, and—in the case of roots—from different habitats and depths in the soil. The location in the tree, its size, and the type of habitat are indicated in figures 1-5. In these figures the term “stems young trees 1 mm.” describes the inner wood 1 mm. from the pith in the stems of small trees approximately 0.5 m. tall. Data for the inner wood of branches, 1 mm. from the pith, were obtained from both the distal parts of old branches near the ground and from smaller branches at the tops of mature trees. Data for the outer wood of branches, 1-2 cm. from the pith on the upper side and 1-3 cm. from the pith on the lower side, were secured from the proximal portions of large branches of old trees. In the case of the “stem tips” the inner wood 1 mm. from the pith at the tops of the trees was studied, both of “medium” trees 3 m. tall and of “old” trees 10 m. or more tall. “Stems old trees 1-3 dm.” describes the outer wood 1-3 dm. from the pith at the base of old stems.

In the case of roots the habit varied with the environment. The root systems in wet soils were flat and platelike, with all roots confined to the top layers of soil, whereas in well-drained sandy or gravelly soils there were in addition roots which extended deep into the ground. The latter are described as vertical roots to distinguish them from the widely spreading lateral roots located close to the surface. None of the roots included in the designation lateral were deeper than 6 inches, and the majority were only 2-3 inches deep. The lateral roots were collected from both wet and dry soils, “sand hill” indicating well-drained sandy soil on hilltops, and “cedar swamp,” low-lying wet soils. Both the inner wood 1 mm.

after exposure. The various types of roots were examined at distances of 1-3 m. from the bole. All lateral, vertical, and exposed roots were growing from mature trees. A few roots of small trees 0.5 m. tall were studied and these are described in the figures as "roots young trees."

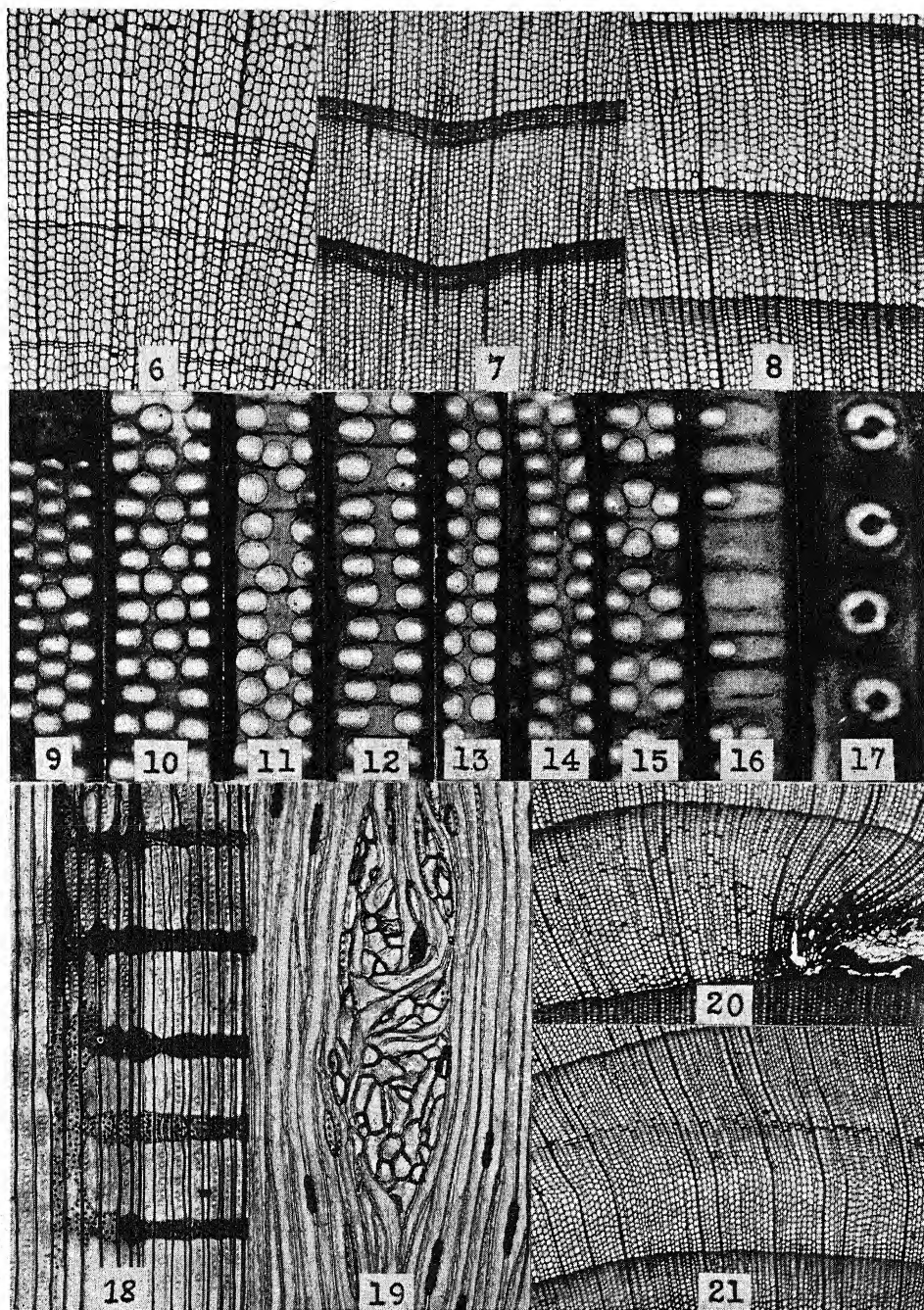
Usually more than ten specimens of each type of stem, branch, and root were examined for the compilation of the data in figures 1-5 and figures 22-25. In these figures the maximum average (in the specimen with the largest elements) is shown by the upper broken line, the grand average for all specimens by the middle solid line, and the minimum average (in the specimen with the smallest elements) by the lower broken line.

Observations

TRACHEID SIZE

Data on the radial and tangential diameters of tracheids in different parts of the tree are presented in figures 1 and 2. These values were determined from the early-wood tracheids in the growth rings and show the external dimensions of the cells from one middle lamella to the next. The measurements were made from transverse sections and included both wide and narrow cells. Had the determinations of the tangential diameters been made from only the central widest part of each tracheid, as observed in tangential sections, the values would have been 20-25 per cent higher than those in figure 2.

The smallest early-wood tracheids were observed in the inner wood of the stems of small trees, of branches both at the top and near the base of old trees, and of stem tips of both medium-sized and old trees. In the inner wood of these different parts of the tree the tracheids were uniformly small (figs. 1, 2). Outward from the center in both stems and branches the tracheids tended to increase in diameter. In roots the tracheids in the early wood of the inner growth rings were usually much larger than in stems and branches. As shown in figures 1 and 2, they were merely slightly larger in lateral than in vertical roots, and in the former only minor differences were noted between roots in dry and wet soils. This inner wood showed much greater variation among different roots of the same type or roots growing in the same habitat than among different kinds of roots. Outward from the center, however, distinctly different tendencies were noted. In the case of lateral roots growing in sand, the size of the tracheids increased slightly from the inner to the outer wood, whereas in roots growing in swampy soils the size decreased toward the outside. An even more noticeable decrease was observed in exposed roots, where the outer growth rings—laid down presumably after removal of the soil—were stemlike (fig. 7) and the tracheids were much smaller than in the inner wood (fig. 6). The amount of soil moisture did not appear to have had much effect on the texture of the inner wood of lateral roots, but, as already



FIGS. 6-21.—Fig. 6, open type of wood in lateral root. Fig. 7, conspicuous late wood in outer rings of exposed root. Fig. 8, stemlike wood in vertical root 6 dm. deep in soil. Figs. 9-16, various arrangements of pits on radial walls of tracheids in inner wood of lateral roots. Fig. 17, structure of torus. Fig. 18, radial view of newly formed rays in root wood. Fig. 19, tangential view of unusual tracheary ray in stem wood. Fig. 20, diffuse distribution of xylem parenchyma cells in stem wood. Fig. 21, tangential arrange-

pointed out, differences were noted in the outer wood. The influence of site on tracheid caliber in stem wood has been dealt with by HARLOW (6).

In both stems and roots the tracheids in the late wood of the annual rings were much smaller than the early-wood tracheids. The transition ranged from abrupt (figs. 6, 7) to gradual (fig. 8), and the amount of late wood from the minimum of one row (fig. 6) to a considerable portion of the annual ring (fig. 8). In general the smallest amount of late wood occurred in the inner wood of lateral roots and the outermost wood of some old branches, whereas in the outer wood of roots growing in swamps, in the peripheral rings of exposed roots, in the outer wood of some vertical roots, and in the inner wood of most branches and stems, late wood was usually more prominent. There was much variation, however, among different specimens of the same type and often among different rings in the same specimen. For instance, the wood in vertical roots was highly variable, some specimens possessing an open type near the center and stemlike wood in the outer rings (fig. 8), while in others the wood was more or less uniformly intermediate.

The amount of the so-called compression wood, composed of rounded, thick-walled cells, likewise varied considerably. Sometimes the whole or greater part of growth rings in the inner wood of branches and stems was made up of such tracheids, while in other cases these were distributed in zones with sharp or gradual transitions to layers of thin-walled, rectangular tracheids between.

TRACHEID PITTING

The intertracheary bordered pits on the radial walls varied considerably in size and arrangement in different parts of the wood. They were usually largest in the outer rings of old stems and the inner wood of lateral roots, where they averaged 11–14 μ in diameter. In the annual rings the size tended to decrease from the early to the late wood. There was usually only one row of pits on the radial walls of the early-wood tracheids in the inner wood of stems and branches; usually one (but occasionally two) in the outer wood of old stems; and often two or sometimes three in the inner wood of lateral roots, where the tracheids were wider than elsewhere in the tree. When biseriate or triseriate, the pits were generally opposite (that is, in the same horizontal rows), but occasionally they alternated. Crassulae were exceedingly variable but on the whole were rather better developed in roots and the outer wood of old stems than in the inner wood of stems or branches. The greatest variation in the arrangement of pits and in the development of crassulae was found in the inner wood of lateral roots. Here the pits were usually biseriate and opposite, with conspicuous straight crassulae (fig. 12), or grouped with curved crassulae (fig. 15); but sometimes the crassulae were indistinct or absent (fig. 13), and in a few cases the pits alternated and crassulae were lacking (fig. 14). When triseriate, the pits were generally opposite, with or without crassu-

lae (fig. 10), less often mixed, sometimes with straight crassulae (fig. 11), and occasionally alternate, generally without crassulae (fig. 9). Toward the overlapping ends of the tracheids the pits tended to be crowded, but elsewhere were often more widely spaced (fig. 16). The pit apertures were usually broadly oval or round in the early wood and slitlike in the late wood.

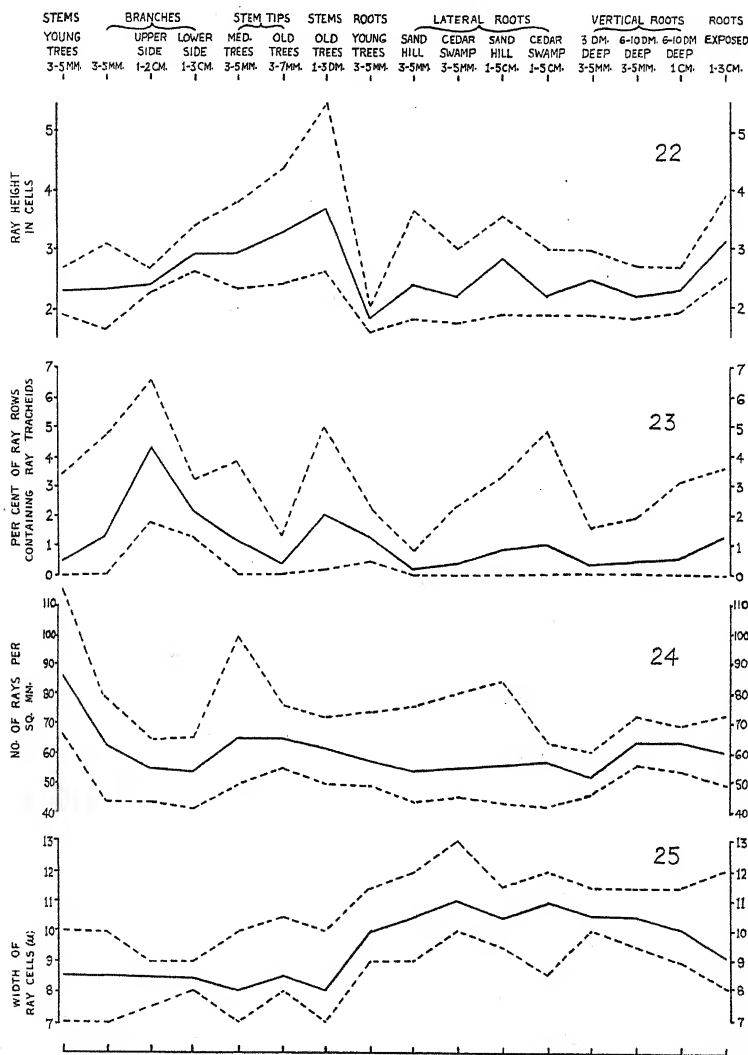
As shown in figures 9-16, tori were not discernible in most of the intertracheary pits. Only sporadically in different parts of the tree were tori recognized. They ranged in size from one-half the diameter to slightly larger than the pit aperture, and had irregular or lacerate margins (fig. 17). The absence or feeble development of the torus was a striking feature of the wood.

Bordered pits in the tangential walls of the tracheids occurred characteristically in the late wood and only rarely in the early wood of the growth rings. In the latter case they were associated with injuries or abnormalities. For example, in two specimens of stem wood certain of the radial rows of tracheids in the early wood of one of the growth rings were composed of cells which were much narrower radially than the typical tracheids nearby. These particular cells resembled late wood tracheids in size and shape, and—like the latter—had pits on their tangential walls. The pits were sporadic, however, and approached in size the pits on radial walls. Tangential pitting also occurred in early-wood tracheids in or near injuries. The usual tangential pits in the late wood varied greatly in size, but generally averaged $5-7\ \mu$ in stems and branches and $7-9\ \mu$ in roots.

The number of pits per crossing field between tracheids and ray parenchyma cells ranged from none to more than fifteen, depending upon size and shape of the ray cell, size of the tracheid, location in the annual ring, and part of the tree. There were, for instance, more pits between tracheids and the vertically elongated or squarish-shaped cells in the first-formed parts of new rays (fig. 18) than between tracheids and the lower, radially elongated cells in the succeeding parts of the rays. A comparison of figures 1, 3, and 5 shows that the most numerous pits occurred in the inner wood of roots, where the tracheids were wider and the ray cells higher than in other parts of the tree, and fewest in the outer wood of old stems and branches, where the height of the ray cells was at a minimum. Outward from the center in stems, branches, and roots the number of pits tended to decrease, as did also the average height of the ray cells. The range in different parts of the tree was considerable, from an average of 5.6 in the inner wood of lateral roots to only 2.6 in the outer wood of old stems. The values shown in figure 3 were determined from rays two or more cells high consisting entirely of radially elongated cells and refer only to the early wood. In each growth ring the number decreased toward the late wood.

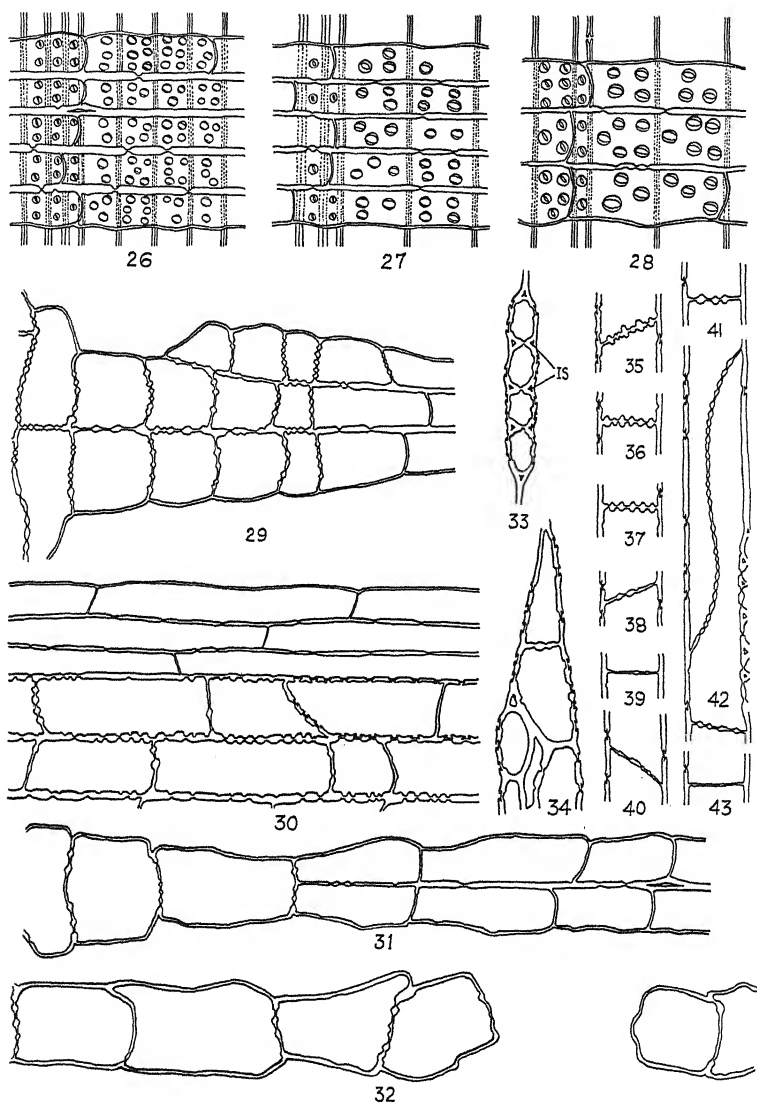
The pit apertures in the crossing fields of the early-wood tracheids were exceedingly variable, ranging from narrowly elliptic with broad borders to round

with reduced or absent borders. Generally the pits with the largest apertures and narrowest borders were found in the inner wood of stems and branches (fig. 26),



FIGS. 22-25.—Variation in different parts of tree: Fig. 22, ray height in cells. Fig. 23, percentage of ray rows containing ray tracheids as determined from radial sections. Fig. 24, number of rays per sq. mm. of tangential section. Fig. 25, width of ray cells in tangential section.

the outer wood of some stems (fig. 27), and the outer wood of certain roots such as those exposed on the surface of the ground. Pits with narrower apertures were observed most often in the inner wood of roots (fig. 28), but in some cases directly



FIGS. 26-43.—Fig. 26, ray structure in inner stem wood. Fig. 27, same in outer wood of old stem. Fig. 28, ray structure in lateral root. Fig. 29, wall structure of cells in ray beginning at pith. Fig. 30, same of cells in unusual ray in old stem wood. Fig. 31, same of cells in ray arising at center in lateral root. Fig. 32, same of cells in newly formed ray in old stem wood. Fig. 33, tangential view of ordinary ray in stem wood showing intercellular spaces (*is*). Fig. 34, tangential view of unusual ray in branch wood showing lack of intercellular spaces. Figs. 35-43, tangential views showing structure of transverse walls of xylem parenchyma cells in outer wood of old stems.

opposite dispositions were found. The orientation of the apertures varied from horizontal to a definite inclination. In the late-wood tracheids of growth rings throughout the tree the apertures of the crossing-field pits were generally slitlike and steeply oriented.

The size of the crossing-field pits varied considerably, even within the same field, but in the early wood averaged about $5-6\ \mu$ in the inner wood of lateral roots, $5\ \mu$ in the outer wood of old stems, and $4\ \mu$ in the inner wood of branches and stems (fig. 4). In general the size tended to increase outward from the center in stems and to decrease outward from the center in roots, and from the early to the late wood in growth rings throughout the tree.

XYLEM PARENCHYMA

Xylem parenchyma cells were variable in distribution. When abundant, as for instance in the vicinity of injuries, they were diffuse (fig. 20) or more or less aggregated in tangential arrangement (fig. 21). When scarce the cells were widely dispersed, sometimes only in late wood, less often in the early wood. Generally xylem parenchyma cells were more abundant in the inner wood, particularly of branches and roots, than in the outer rings.

The transverse walls of the parenchyma cells were occasionally only slightly thickened (figs. 39, 40, 43), but more often were definitely and regularly thickened or beaded (figs. 37, 41) or irregularly thickened (figs. 35, 36). The walls, while usually at right angles to the long axis of the cell (figs. 36, 37, 39, 43), were sometimes oblique (figs. 35, 38, 40), and rarely were almost vertical (fig. 42). Often the transverse walls were somewhat thinner in late than in the early wood of the growth rings, but otherwise no trends were apparent in the amount of wall thickening in different parts of the tree.

STRAND TRACHEIDS

In the material studied these cells were found only in the vicinity of injuries. The elements were made up partly of parenchyma, partly of tracheary cells.

SIZE AND DISTRIBUTION OF RAYS

The majority of the rays were uniseriate, seldom more and usually much less than 1 per cent of the rays being partially biseriate or wider. Their height ranged from one to more than twenty cells. In stems and branches the height tended, on the average, to increase from the inner to the outer wood, attaining a maximum in the outer rings of old stems (fig. 22). There was considerable variation among different specimens, the average in the periphery of old trees ranging from 2.7 cells in one specimen to 5.5 cells in another. In most roots the rays were relatively low, the average in different specimens fluctuating between 1.7 and 3.7

cells, with the grand average ranging between 2.2 and 2.8 cells (fig. 22). The height tended to increase in the outer wood of exposed roots, where the average was 3.1 cells. All these determinations were made from radial sections.

The size and shape of the individual ray cells were exceedingly variable. The cells in the first-formed parts of new rays, particularly those arising in the inner wood, were often vertically elongated (fig. 18), whereas most of the cells in the succeeding parts were lower and lengthened in the radial direction. The length of the latter was greater in the early than in the late wood of each annual ring, and was usually greater in the outer wood of old stems than in the inner wood. The average height of these radially elongated cells fluctuated considerably in different specimens, but was generally higher in roots than in stems or branches (fig. 5), and in both roots and stems tended to decrease outward from the center. In lateral roots the average height decreased from $27\ \mu$ near the center to $22\ \mu$ in the outer wood and in stems declined from $22\ \mu$ near the pith to $18\ \mu$ in the periphery. The average width of the ray cells was $10\text{--}11\ \mu$ in roots and approximately $8\ \mu$ in stems and branches (fig. 25).

It has been shown by ESSNER (4) for several conifers that the number of rays in the stem tends to decrease outward from the center, but, as SHIMAKURA (9) has demonstrated, this tendency may be modified by the width of the succeeding growth rings. In the material of *Thuja occidentalis* examined by the writer the average number of rays dropped from 87 per sq. mm. in the inner wood to 61 in the outer wood of old stems (fig. 24), and in branches decreased from 63 near the center to 55 and 53 in the outer rings. There was also a decrease from the base to the upper levels in the stem. At the same distance from the pith there were 87 rays per sq. mm. in the stem bases of small trees as compared with an average of 65 per sq. mm. in the stem tips of trees 3 m. and 10 m. or more tall. JACCARD (7) has described similar distributions in other conifers. In roots no particular tendencies in distribution were noted. The rays as a rule were of approximately similar distribution in both inner and outer wood (fig. 24). Much greater variation was observed among individual roots of the same kind than among different parts of the root or among roots in different habitats or depths in the soil. In this lack of correlation *T. occidentalis* differed markedly from certain other conifers (2).

In addition to the ordinary uniseriate rays just described there were sporadic rays which differed strikingly in size, structure, and distribution. These unusual rays were sometimes much higher than the ordinary ones; their width ranged from one to occasionally ten or more cells; the individual ray cells were of many diverse shapes; and in some instances parts of the ray were largely tracheary (fig. 19). They originated from uniseriate parenchymatous rays usually traceable to the pith or primary xylem, and toward the outside broadened and subsequently

followed different courses of development. Some connected with adventitious roots, others split up into smaller rays, and yet others became gradually reduced in size so as to resemble ordinary rays again. They were found throughout the aerial parts of the tree, but their distribution was erratic (3). The most frequent occurrence was observed in the outer wood on the lower side of large branches, where in one instance they attained an average frequency of 65 to the sq. cm. (as compared with 5300 ordinary rays per sq. cm.). They were seldom found in roots.

RAY STRUCTURE

The ordinary uniseriate rays which began at the pith or in the early secondary xylem were usually parenchymatous throughout. Rays of later origin, particularly those arising in the outer wood of stems and branches, generally contained ray tracheids in their first-formed parts; but in the succeeding parts, where the ray height increased, the cells became parenchymatous. Sometimes such recently formed rays containing ray tracheids were applied to the margins of older parenchymatous rays and formed marginal tracheary rows along the latter.

Because ray tracheids were usually restricted to new rays or marginal rows formed as already described, they did not constitute a large proportion of the tissue in the ordinary uniseriate rays. The most frequent occurrence was in the outer wood of branches and stems (fig. 23), where on the average 2-4 per cent of the ray rows contained ray tracheids, but even here their distribution was erratic. For instance, in old stems the range was from nearly zero in one specimen to more than 5 per cent in another. Moreover, these rows were not exclusively tracheary but in some cases contained only some ray tracheids intermixed with parenchyma cells, so that the actual proportion of ray tracheids in terms of parenchyma cells was even less than indicated in figure 23. These determinations were made from radial sections where ray tracheids were more obvious than in tangential sections. In roots ray tracheids were scarce. Their most frequent occurrence was in the outer wood of certain exposed roots and of roots growing in swamps, where the wood was stemlike in other features, but even here the average was only about 1 per cent (fig. 23). In the inner wood ray tracheids were yet more infrequent. A noteworthy feature of the distribution of ray tracheids in both roots and stems was the wide variation among different specimens of the same type.

The ray tracheids were of many diverse shapes, especially in the earliest-formed parts of the new rays; but farther along in the ray, or where the new rays were applied to the margins of older ones, the cells tended to be elongated radially. The bordered pits on the radial walls adjoining tracheids were generally relatively large, averaging about $8\ \mu$, but in some instances graded down to $3\ \mu$. Some ray tracheids had only large pits; others exhibited mixtures of large and small pits; and a few, particularly those located nearest the point of ray origin, sometimes

possessed only small pits. Occasionally the pits were only dimly outlined and the walls were thin, the appearance suggesting that the protoplasm had disappeared before wall thickening had been completed. Bordered pits on the horizontal walls in contact with ray parenchyma cells ranged from few to numerous, and the number of pits on the tangential walls between ray tracheids also varied considerably. Sometimes these walls lacked pits.

The preceding descriptions apply only to ray tracheids in the ordinary uniseriate rays. In addition to the latter, however, there were sporadic unusual rays which differed markedly in size and structure. They originated from uniseriate parenchymatous rays but subsequently underwent strikingly different courses of development (3). The tracheary cells in these unusual rays were highly variable in number, position in ray, shape, size, and structure. They fluctuated from few—sometimes interspersed among thick-walled parenchyma cells in the central portions of the ray (fig. 30)—to many, comprising the bulk of the ray (fig. 19). Their shape ranged from radially elongated to rectangular, squarish, vertically elongated, and varied angular forms (fig. 19). Where the unusual rays were only one or two cells wide and ray tracheids were appearing for the first time, these cells were often radially elongated or somewhat squarish in shape, and resembled typical ray tracheids with bordered pits commonly on all walls but sometimes absent from tangential ones. As the unusual rays widened these cells were sometimes followed by others in which there was progressive lengthening in the vertical direction, so that some of the tracheary cells came to resemble short fusiform elements. As in the vertical tracheids comprising the wood, the bordered pits on the radial walls of these cells were often large, occasionally attaining the size of intertracheary pits, and faint crassulae were discernible above and below. The tangential walls often lacked pits. Where such series of transitional types occurred it seemed clear that certain of the ray initials had elongated until they acquired the stature of short fusiform initials. In some cases this continued enlargement resulted in a splitting up of the ray into separate smaller units, a process which sometimes was aided also by penetration of adjoining ordinary fusiform initials into the ray. In other instances the ray initials, after having elongated, gradually shortened, and some ceased dividing so that the ray tissue was reduced in quantity. Other unusual rays, instead of becoming subdivided into smaller ones, connected with adventitious roots, the root meristems apparently having been organized in the phloem or cambial portions of the rays. In the wide parts of these rays, where the vascular tissues of the newly formed root were first appearing, the tracheary cells showed gradations in size, shape, and structure from more or less typical ray tracheids in the ray proper to primary xylem tracheids in the new root.

Ray parenchyma cells in the ordinary uniseriate rays typically had thin tangential walls and thick horizontal and radial walls (figs. 26–28). The latter were

described by BAILEY and FAULL (1) as thickened primary walls bearing deeply depressed primary pit-fields. The tangential walls were usually thin (figs. 26-28) but sometimes were definitely thickened, particularly near the pith (fig. 29), less often near the center of roots (fig. 31), and rarely in the first-formed parts of new rays arising in the outer wood (fig. 32). The horizontal walls were generally thick, with widely spaced pit-fields (figs. 26-28), but sometimes were comparatively thin, especially in new rays arising near the center of roots. As a rule the horizontal walls of the cells in the older parts of the rays were slightly thinner in the outer wood of old stems and the outer wood of some roots than elsewhere in the tree. Indentures, or recessions in the horizontal wall adjoining the point of contact with the tangential wall, have been described by PEIRCE (8) as regularly present and pronounced in *Thuja*, but in material examined by the writer were variable. They were usually present in the outer layers of old stems but occasionally were shallow or absent; they were often well developed in the inner wood of stems and branches but at other times were not discernible; and in the inner wood of some lateral roots they were frequently lacking or only feebly developed.

The ray parenchyma cells usually retained their protoplasm for several years, but in some cases, especially in the first-formed parts of rays or where a row in an old ray had ceased, the protoplasm disappeared early. The walls of these cells were often thinner than in the living cells, and the number of pits in the crossing field was occasionally noticeably reduced. In one or two instances only an outline or "ghost" was discernible, and the adjoining tracheids lacked pits in the crossing field.

Yet other types of parenchyma cells were observed, particularly in some of the sporadic unusual rays. Here certain of the cells, as shown in the lower two rows in figure 30, had thick lignified walls with numerous pit-fields. The thickness of the tangential walls especially was in marked contrast to the uniformly thin condition in the corresponding walls of the more typical ray cells in the upper three rows. The protoplasts of the thick-walled cells disappeared early in some instances but in others remained as long as in the typical ray parenchyma cells. Intercellular spaces between the thick-walled cells and the adjoining tracheids were generally much reduced or absent (fig. 34), while those associated with typical ray cells were conspicuous (fig. 33). Where the unusual rays attained their maximum width the range of structural variability in the parenchyma cells was even greater. From cells with thick, knoblike, heavily lignified walls there were gradations to cells with uniformly thin, unlignified or slightly lignified walls on all sides. Some of the cells were dead; others living. The cell shape ranged from round and distended to irregular and angled, and occasionally some of the thin-walled cells had collapsed. The diverse types and shapes of cells occurred in various proportions in different rays and in different parts of the same ray. Intercellular spaces were variable.

Discussion

Some features of the wood of *Thuja occidentalis* varied considerably from one specimen to another without evidence of definite trends in any parts of the tree. In other features distinct tendencies were apparent in some parts of the tree though perhaps not in other parts. For instance, the tracheids increased in size outward from the center in stems but not in roots, and as a rule were noticeably larger in roots than in stems; the number of rays decreased outward from the pith in stems but in roots remained relatively constant; the ray height increased outward from the center in stems and in certain types of roots, and in the inner wood was approximately the same in roots as in stems or branches; the height of the individual ray cells and the number of pits per crossing field decreased toward the outside in both stems and roots. Certain of these tendencies have been noted in other genera, and they may prove to be general among the conifers. For example, the decrease in number of rays outward from the pith has been noted in several genera (4, 5). In other cases, however, the degree or even direction of trend varies with the genus. It has been shown in *T. occidentalis* that the average ray height in the inner wood of root and stem is similar, whereas in some of the Abietineae the rays in certain types of roots are definitely higher than in stems of like size (2). Preliminary investigations have indicated that divergency in trend may occur even among the Cupressineae. Hence it is unwise to assume that tendencies in anatomical variation noted in one genus necessarily occur in another. Variability in structure and divergency in trend render imperative the use of homologous parts of the tree when comparing or contrasting the woods of different conifers.

Although many specimens of the various other Cupressineae were studied, neither sufficient quantities nor variety of material were available to allow satisfactory comparisons with *T. occidentalis*. For this reason no attempt will be made to select characters useful for the identification of *Thuja* wood or its separation from other Cupressineae. PEIRCE (8), in his descriptions of *Thuja*, referred to the large size of the apertures in the crossing-field pits as being unique, and described indentures in the horizontal walls of the ray cells as "regularly present and pronounced." Both these features were often well developed in stems but in some specimens and in other parts of the tree were variable, the indentures particularly being too inconstant to be diagnostic. SLYPER (10) in his key separated *T. occidentalis* from *T. gigantea* and *T. standishii* on the absence of thickenings on the transverse walls of the xylem parenchyma cells. He stated that the walls in *T. occidentalis* were nearly smooth, whereas the writer found them to be sometimes smooth but more often definitely thickened or beaded. Because of such variability, attempts to differentiate closely similar woods by utilizing only single anatomical characteristics of the type here mentioned seem futile. If identifications are to be positive, not single but rather combinations of characters must be used.

Summary

1. Studies of samples from different parts of the tree, from trees of different sizes, and—in the case of roots—from roots growing under different environmental conditions, revealed much variation in the structure of the secondary xylem.
2. Some features usually recognized as variable, namely, size of tracheids, number and size of crossing-field pits, height and distribution of rays, and height and width of ray cells, were found to vary widely; but in some cases definite trends were recognizable in different parts of the tree.
3. Other characters more generally regarded as diagnostic, such as the arrangement of the intertracheary pits on the radial walls of tracheids, the degree of development of the torus, the presence or absence of crassulae, the size and shape of the apertures in the crossing-field pits, and the thickness of the walls of ray and wood parenchyma cells, also varied greatly, often within the same piece of wood.
4. In view of such variability it is obvious that caution must be exercised in the selection of diagnostic characters for the identification of woods.

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LITERATURE CITED

1. BAILEY, I. W., and FAULL, ANNA F., The cambium and its derivative tissues. IX. Structural variability in the redwood, *Sequoia sempervirens*, and its significance in the identification of fossil woods. Jour. Arnold Arb. 15:233-254. 1934.
2. BANNAN, M. W., Variability in wood structure in roots of native Ontario conifers. Bull. Torrey Bot. Club 68:173-194. 1941.
3. ———, Vascular rays and adventitious root formation in *Thuja occidentalis*. Amer. Jour. Bot. 28:457-463. 1941.
4. ESSNER, B., Über den diagnostischen Werth der Anzahl und Höhe der Markstrahlen bei den Coniferen. Abhandl. Naturf. Ges. Halle 16:1-33. 1883.
5. FISCHER, H., Ein Beitrag zur vergleichenden Anatomie des Markstrahlengewebes und der jährlichen Zuwachszonen im Holzkörper von Stamm, Wurzel und Aesten bei *Pinus abies* L. Flora 43:263-294; 302-309; 313-324. 1885.
6. HARLOW, W. M., The effect of site on the structure and growth of white cedar, *Thuja occidentalis* L. Ecology 8:453-470. 1927.
7. JACCARD, P., Über die Verteilung der Markstrahlen bei ben Coniferen. Deutsch. Bot. Gesell. 33:492-498. 1915.
8. PEIRCE, A. S., Systematic anatomy of the woods of the Cupressaceae. Tropical Woods 49:5-21. 1937.
9. SHIMAKURA, M., The height and number of rays in some coniferous woods. Bot. Mag. Tokyo 50:438-447. 1936.
10. SLYPER, E. J., Bestimmungstabelle für rezente und fossile Coniferenhölzer nach mikroskopischen Merkmalen. Recueil Trav. Bot. Néerland 30:482-513. 1933.

GEMMAE-CUP PRODUCTION IN MARCHANTIA POLYMORPHA AND ITS RESPONSE TO CALCIUM DEFICIENCY AND SUPPLY OF OTHER NUTRIENTS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 532

PAUL D. VOTH

(WITH SIX FIGURES)

Introduction

Excellent adaptability to nutrient supply and photoperiod has been reported recently for *Marchantia polymorpha* L. (15). Nutrient triangles with relatively small numbers of positions were adequate to effect distinctive growth responses when calcium, phosphate, and nitrate supply was systematically varied. The present study deals principally with the effect of relatively smaller proportions of the six common ions, with particular reference to lower limits of calcium and phosphate supply and to larger concentrations of nitrates. This is accomplished by employing triangles each having fifty-five positions. Similar nutrient triangles have been employed by SCHREINER and SKINNER (11), TRUE (14), and others.

As previously, records were made of changes in gross appearances of the plants, increases in total area, accumulation of dry weight, and formation of gemmae cups. From the data secured it is possible to suggest the relative proportions and concentrations of the commonly used nutrient salts conducive to the greatest vegetative development of this plant. The anatomical details of the apices of plants growing in nutrient solutions deficient in calcium may give clues to the function of calcium in meristematic and differentiating cells and tissues. Comparison of the total number of gemmae cups on antheridial and archegonial thalli seems to offer possibilities in the identification of the sex of vegetative plants of *M. polymorpha*.

General methods

The strains of plants used in this investigation are from the clones used in experiments 8 and 9 (15) and originated as follows:

Culture A: One male plant selected from plants growing in the greenhouses of the University of Chicago, May 16, 1939.

Culture B: One female plant similarly selected, May 15, 1939.

From the new tips which form dichotomously as the plant grows, these clones have been perpetuated uninterruptedly.

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All experimental work was conducted in the greenhouse, using the culture method of placing six plants on a disk of glass cloth supported by a glass rack and placed in the open half of a moist chamber. Each culture was supplied with about 200 cc. of fresh solution daily (15). The plants and apparatus received a daily pressure-rinsing with distilled water, which also served to remove excess algae from the surface of the glass cloth and from the water line in the moist chamber.

All plants grew for 32 days, September 24 to October 27, 1940. Supplementary light was supplied with 200-watt Mazda filament bulbs in reflectors, suspended about 33 inches above the plants. A maximum of 200 foot-candles directly underneath the reflectors and more than 100 foot-candles between the lights at the level of the table top was recorded at the beginning of the experiment, when reflection from the glass cloth was maximum and plant size minimum. These lights were on from sundown until 2:00 A.M., a total photoperiod of 18 hours in each 24-hour period.

Because of experience gained previously, the phosphate supply was considerably decreased, the nitrate supply was increased, and the other ions were given in relatively the same proportions as in experiments 8 and 9. The molar concentration of each ion as well as the amount of each salt used is shown in table 1.

Of the fifty-five units constituting each triangle, only thirty-seven were actually used in the present study. Since the omission of a single ion resulted in striking symptoms in previous experiments (particularly K, Ca, NO_3 , and PO_4), many positions along the sides of the triangles were not repeated. The positions actually employed in this study are indicated by squares and the unused ones by circles in figure 1. In a similar triangle, anions were varied and cations remained constant; the NO_3 position was at the top, PO_4 at the lower left, and SO_4 at the lower right.

Male and female plants of cultures A and B, respectively, were grown in separate moist chambers, but the two cultures were always adjacent to facilitate changing of solutions and to provide as comparable a habitat as possible for both sexes.

As in experiments 8 and 9, gemmae cups were counted, all plants were photographed by groups, areas were determined photometrically, fresh weights were recorded, one plant from each dish was preserved in formalin-acetic acid-alcohol preservative, and the remaining plants were weighed when dry. The percentage dry weight of five plants was used to calculate the dry weight of six plants. The present investigation is designated experiment 10.

Investigation

RESPONSE TO OMISSION OF IONS

CATION TRIANGLE.—Each *Marchantia* cutting, when planted on September 24, averaged 3 sq. cm. in area. Plants on positions 10, 28, 1, and 55 received no calcium, and the latter two were given no magnesium and no potassium, respectively. These cultures grew only slightly, with a maximum final area of only about three

TABLE 1

CONCENTRATION OF SALTS IN THE SIX STOCK NUTRIENT SOLUTIONS USED IN MAKING UP 74 OTHER COMBINATIONS. TO EACH SOLUTION WAS ADDED 0.2 P.P.M. OF MnSO_4 , ZnCl_2 , AND $\text{Na}_2\text{B}_4\text{O}_7$, AND ALSO 0.02 P.P.M. OF FeSO_4 . THE NINE SALTS ARE IDENTICAL WITH THOSE OF EXPERIMENTS 8 AND 9 (15)

MOLAR CONCENTRATION	SALT CON- TENT (CC. OF 0.5M SOLUTION) PER L.	MOLAR CONCENTRATION	SALT CONTENT (PER LITER)	MOLAR CONCENTRATION	SALT CONTENT (PER LITER)
CATIONS VARIED					
K SOLUTION		CA SOLUTION		MG SOLUTION	
K.....0.0054	Ca.....0.0027	Mg.....0.0029
NO_3 ...0.0032	6.40*	NO_3 ...0.0032	3.20 cc. of 0.5M	NO_3 ...0.0032	3.20 cc. of 0.5M
PO_4 ...0.0004	0.80†	PO_4 ...0.0004	0.0504 gm.‡	PO_4 ...0.0004	0.0698 gm.§
SO_4 ...0.0009	1.80	SO_4 ...0.0009	0.1550 gm.	SO_4 ...0.0009	1.80 cc. of 0.5M
ANIONS VARIED					
NO_3 SOLUTION		PO_4 SOLUTION		SO_4 SOLUTION	
NO_3 ...0.0051	PO_4 ...0.0042	SO_4 ...0.00255
K.....0.0009	1.80*	K.....0.0009	1.80 cc. of 0.5M†	K.....0.0009	0.90 cc. of 0.5M
Ca.....0.0012	2.40	Ca.....0.0012	0.3026 gm.‡	Ca.....0.0012	0.2066 gm.
Mg.....0.0009	1.80	Mg.....0.0009	0.1570 gm.§	Mg.....0.0009	1.80 cc. of 0.5M

* As KNO_3 . † As KH_2PO_4 . ‡ As $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$. § As $\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$, etc.

times the original. A smaller final area than the original may be attributed to disintegration of the dead plants (solution 55). After 4-6 days all plants in cultures deprived of calcium became black tipped and finally died, except a few which regenerated new thalli from the ventral surface of the older plant. Plants on solution 55, lacking calcium and potassium, blackened soonest and were smallest in area.

When potassium is omitted from nutrient solutions, as in positions 49, 52, 46, and 55 (with the latter two also lacking magnesium and calcium, respectively), the growth responses are as follows: Plants on solution 55 respond to the omis-

sion of calcium after a few days. After 18 days, position 46 still possesses small plants (fig. 2), but the characteristic tan color of the older thallus parts—especially along the margins of the wings near the tip—indicates a lack of potassium.

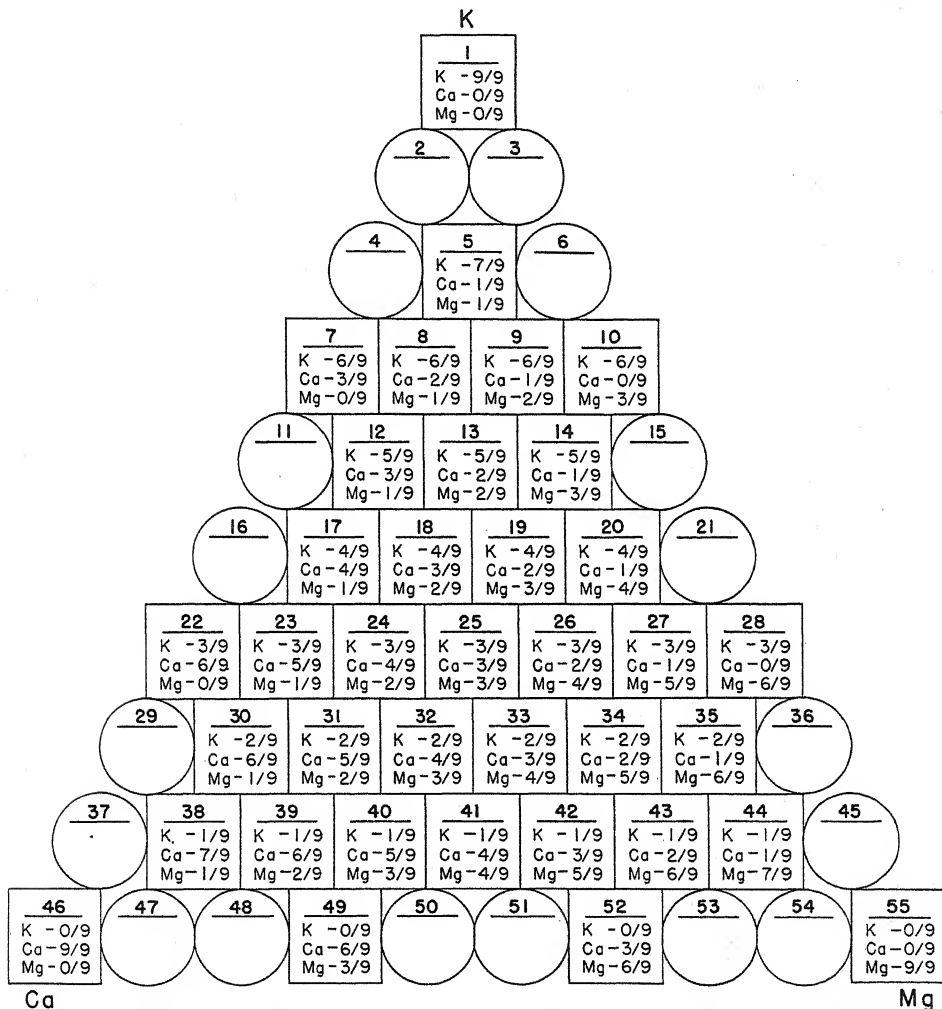


FIG. 1.—Cation triangle showing method of combining stock solutions, on basis of ninths, to secure a range of fifty-five solutions varying in cations and constant for anions. Each apical position lacks two cations; each side of the triangle, one; and the center is supplied with all three in varying proportions. A similar triangle for varying anion combinations was employed: NO_3 at top, PO_4 at lower left, and SO_4 at lower right.

The remaining two cultures lacking potassium develop larger plants without conspicuously brittle margins but with the light brown color in the basal portions of the plants.

The omission of magnesium (solutions 7, 22) affected the total area as well as the dry weights of the plants adversely, but in appearance these plants resembled adjoining cultures receiving a full complement of nutrient ions. The cultures lack-

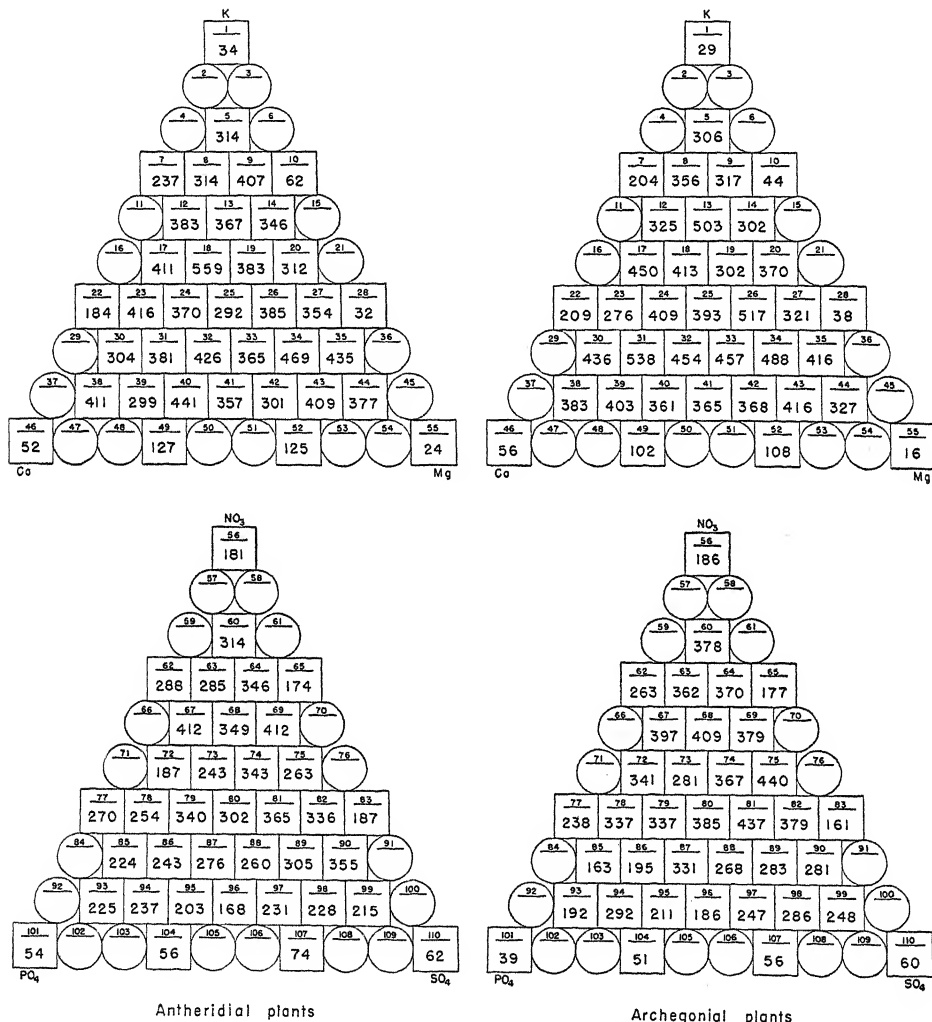


FIG. 2.—Area in square centimeters of six plants growing in each nutrient combination. Two triangles at left are male plants; at right, female.

ing magnesium and another cation responded according to the pattern usual for lack of calcium or potassium (solutions 1 and 46, respectively).

ANION TRIANGLE.—When planted on September 25, each cutting averaged 3 sq. cm. in area. Plants growing in solutions containing no nitrate (101, 104, 107), no phosphate (56, 65, 83), or both (110), developed darker midribs; and scales,

rhizoids, and lower epidermis became red after about 10 days. In all these positions algae were nearly absent from the surface of the glass cloths and of the solutions during the entire experiment. After about 2 weeks the plants lacking nitrates failed to gain in size, remained relatively narrow, produced practically no new gemmae cups, and forked dichotomously only infrequently, so that apical growing points were relatively few in number. The color of the upper surface of the plants became more yellowish as time went on.

The omission of phosphorus resulted in a different response after 2 weeks. Plants possessed a very dark midrib region, and the intensely green color of the upper surface was superimposed on the red color of the under surface. Dichotomy occurred frequently, giving the plants a rosette-like appearance. The plants were wider than average, and gemmae cups were very numerous (fig. 6). Another characteristic of plants lacking phosphorus was the tendency to be closely appressed to the glass cloth. Whether this is a phototropic or a geotropic response, or influenced by chemical or by mechanical stimuli, has not been determined. Only two antheridiophores formed on plants growing in the entire anion triangle—one each on solutions 65 and 69, the former lacking and the latter low in phosphorus.

Omission of sulphate from the solutions failed to alter the gross appearance of the plants and influenced dry weights only slightly (fig. 3). There may have been sufficient sulphur present in the atmosphere, since no special precautions were taken to exclude greenhouse air from the cultures.

RESPONSE TO COMBINATIONS OF ALL IONS

CATION TRIANGLE.—Plants in the twenty-eight positions in the center of the triangle all continued active growth during the entire period. As in previous experiments, the majority of cultures surrounding the exact center (solution 25 in this experiment) were larger in area, or heavier on the basis of dry weight, or both, when compared with the central position. This observation possibly has no great significance until additional data are available. Dry-weight accumulation by both sexes was greatest in position 34 and had a nutrient supply of $\frac{2}{3}$ K, $\frac{2}{3}$ Ca, and $\frac{5}{8}$ Mg solutions. Each triangle of fifty-five possible positions was treated as a unit in assigning rank. With only thirty-seven positions in use, the greatest dry weight accumulation is designated as rank 1 (position 34 in the cation triangles supporting male and female plants, respectively) and the least dry weight as rank 37 (position 28 in the cation triangle of male plants and position 55 in the cation triangle of female plants—both positions from which calcium is absent). The uppermost triangles in figure 3 show that within the central part of the cation triangle the distribution of rank is fairly diverse, indicating that K, Ca, and Mg may be present in rather wide ranges of concentrations and combinations and still be suitable for healthy growth of *Marchantia*.

ANION TRIANGLE.—Of the twenty-eight centrally located positions, plants in the row receiving $\frac{1}{5}$ PO_4 (solutions 60, 64, 69, 75, 82, 90, and 99) appeared most robust after the first 2 weeks, with the exception of plants on solution 99. Plants

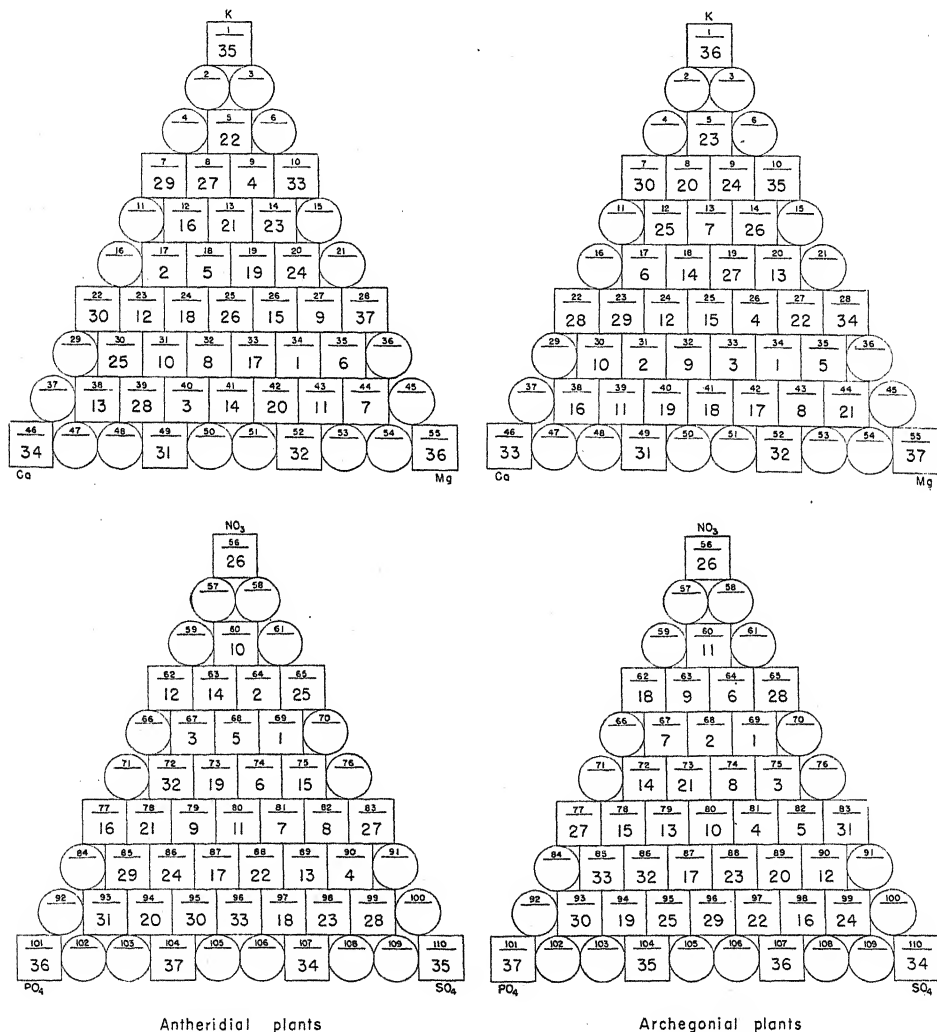


FIG. 3.—Rank on basis of dry weight. Two triangles at left represent male plants; at right, female. Each triangle is a unit for ranking. Rank 1, greatest dry weight, is in same position (no. 34) in cation triangle for male and also female plants. Similarly, rank 1 in anion triangles is in same position for both male and female plants (no. 69).

on solutions 64 and 69 made observably greater growth during the entire growing period, and on the basis of dry weight the latter ranked highest among male and female cultures in the anion triangle (fig. 3). Toward the base of the triangle,

where proportions of nitrates decreased progressively, plants were correspondingly smaller (fig. 2) and had the lowest final dry weights of the complete nutrient plants (fig. 3). Male as well as female plants accumulated the greatest dry weight in position 69, with a solution mixture of $\frac{5}{9}$ NO_3 , $\frac{1}{9}$ PO_4 , and $\frac{3}{9}$ SO_4 . When all six ions were present and anions were varied, increased growth in area and greater accumulation of dry weight were definitely correlated with increased nitrate and decreased phosphate supply, even though the former was greatly increased and the latter decreased in the stock solutions of this experiment. These relationships are summarized in table 1 and figure 3.

RESPONSE TO SMALL AMOUNTS OF CALCIUM

After 24 days some of the plants in the row of the cation triangle receiving only 1 part of calcium to a total of 8 parts of potassium and magnesium began to develop black tips (fig. 4). Often the wings near the apical notch became black first, followed by darkening of the region of meristematic activity; or at times the region of the apical cell(s) suddenly became dark. A V-shaped zone soon developed progressively in a posterior direction, as shown in a plant tip near the upper center of figure 4, 20♂. On many plants the blackened areas became watery. By the end of the experiment, a few days after the first symptoms were noted, many of the blackened tips were dry, curled upward, and were brittle (fig. 4). No correlation seems to exist between this sudden death of the terminals and the factors which were obviously varied—the concentrations of potassium and magnesium. Since the only common factor in all these cultures was a $\frac{1}{8}$ Ca supply, it may be assumed that the low concentration of the calcium ion is responsible for these symptoms. Injury was observably greatest in male and female plants growing in solution 5. The following solutions are listed in the order of decreasing injury to the respective *Marchantia* cultures: 5♂ and 5♀ > 9♂ > 35♂ and 35♀ > 20♀ > 27♂ > 20♂ > 44♂ and 44♀. No external evidence of "burning" was noted in plants growing in solutions 27♀, 9♀, 14♂, and 14♀. In all the preceding cultures a $\frac{1}{8}$ proportion of calcium with reference to the other cations in solution is equivalent to a calcium concentration of 0.3 millimols per liter, or 12 p.p.m.

ANATOMICAL EFFECTS OF CALCIUM DEFICIENCY

When calcium is absent or deficient in any particular plant, the anatomical effects on the cells and organs of that plant often are characteristic enough to be diagnostic for this element. Death of the stem apex (3, 5, 6, 7, 8), abnormalities and death of root tips (1, 4, 7, 8, 12, 13), hooking downward of the tip of young leaves in the bud (5), and yellowing of the youngest leaves (7) followed by necrosis of leaf tips and margins (5, 6) have all been reported for several flowering plants. Premature vacuolization and eventual loss of all cytoplasm in the elongating cells

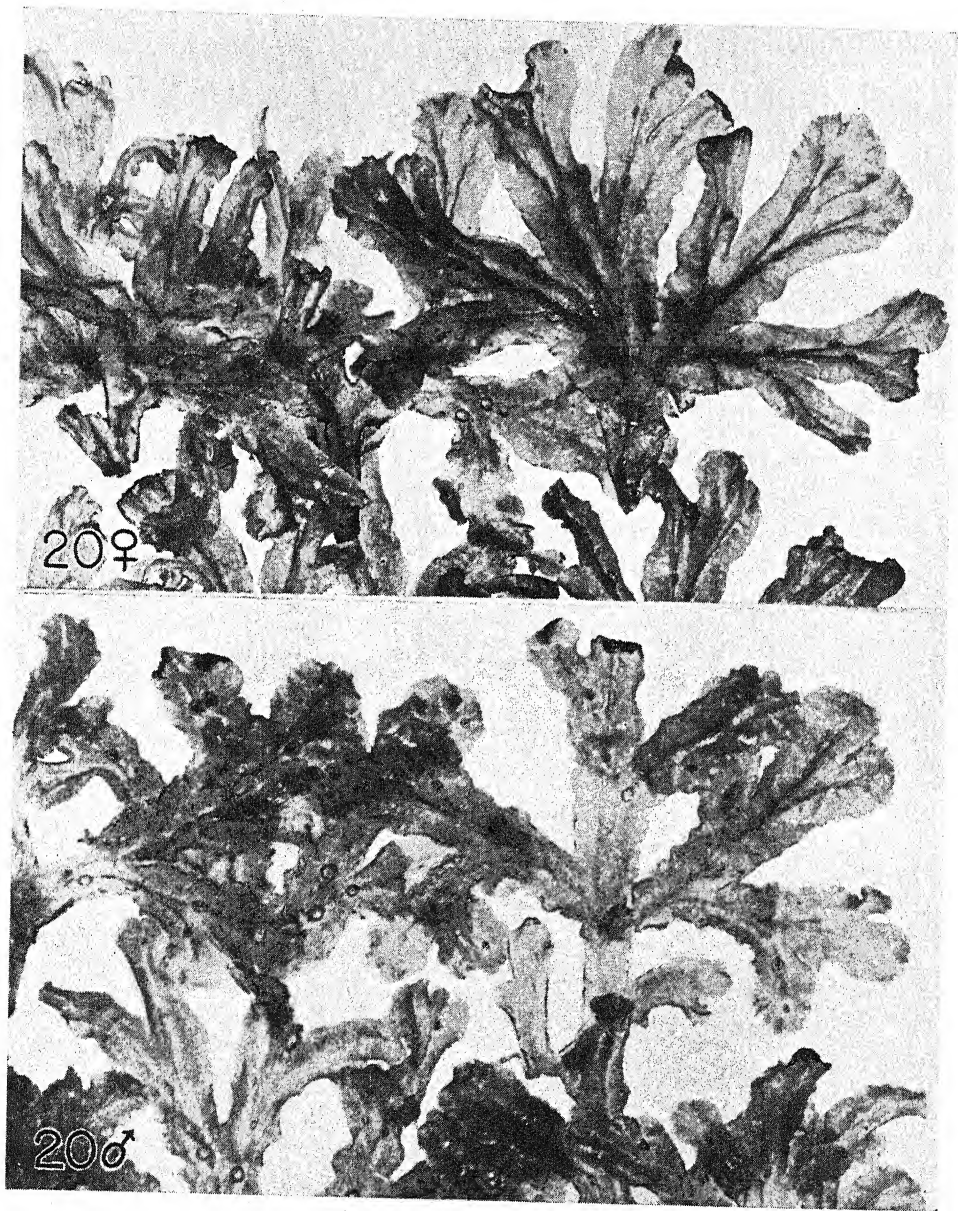


FIG. 4.—Portions of archegonial plants (top) and antheridial plants (bottom) of *M. polymorpha* growing on nutrient combination 20, with Ca supply of $\frac{1}{8}$. Several plants of both sexes exhibit blackened tips, most of which turn upward. $\times \frac{3}{8}$.

of the root tips have been reported for Japanese millet, rape, buckwheat (4), wheat, corn (1), pea (12, 13), and other plants. In rape, cells of the cortex of the stem were large and the stele relatively small when compared with the cortex. In buckwheat the situation was reversed. A similar diversity of response was reported in the length of the palisade cells when calcium is withheld from these two plants (4). Roots of peas growing in the absence of calcium lack resistance to overcome infection by microorganisms (13).

When *Spirogyra* and *Zygnema* are grown on minus-calcium solution, the chloroplast loses its conspicuous lobing, becomes narrow, and soon lacks starch. Excessive vacuolization was followed by general cytoplasmic disorganization, although even after 6 weeks nuclear division was reportedly unaffected but the wall between newly formed nuclei failed to form. After 2 months of calcium absence the apical cell of a *Spirogyra* filament was the first to possess a disorganized chloroplast and to contain a dark substance related to the lecithins (9).

The responses of the gametophyte of *Marchantia* to deficiency or lack of calcium were investigated in detail anatomically. Thallus tips from plants receiving only $\frac{1}{4}$ Ca (solutions 5, 9, 14, 20, 27, 35, and 44), as well as tips from initial control plants growing on a complete nutrient stock solution, and a few tips from cultures surrounding the low-calcium triangle positions, were imbedded in paraffin, sectioned at 7 μ , and stained in Flemming's triple stain. Nearly all tips were sectioned longitudinally. Oblique and transverse sections of the tip confirmed observations made on longitudinal ones. Transverse sections of older parts of the plant are of less value in this phase of the study, since nearly all cells a few millimeters back of the growing tip are mature and practically devoid of cytoplasm and much resemble cells of healthy plants. The walls of the cells of the older portions often stain intensely with methyl violet when the cells of the tips of the same lobe are necrotic. DAY (2) found only slight variation between transverse sections of pea roots taken from healthy and calcium-deficient plants, but the amount of elongation was distinctive.

Plants growing as initial controls on a complete nutrient solution possess an apical growing region characterized by much meristematic activity. The apical cell is not clearly distinguishable from many of the surrounding cells (fig. 5, control ♀). The meristematic zone is several cells wide and merges gradually into the zone of cell enlargement and maturation. Cells and air chambers of the dorsal photosynthetic layer differentiated gradually from the surface layers of the apical meristem (fig. 5, control ♀). In the same treatment ($\frac{1}{4}$ Ca supply) not all plants responded alike; some were entirely necrotic whereas others showed slight injury. As a result, tips taken at random might not represent the state of an entire culture accurately. Many sections from cultures showing a deficiency of calcium possessed degenerating cells (fig. 5, 14 ♀, 9♂, 35 ♀). Longitudinal sections of black-

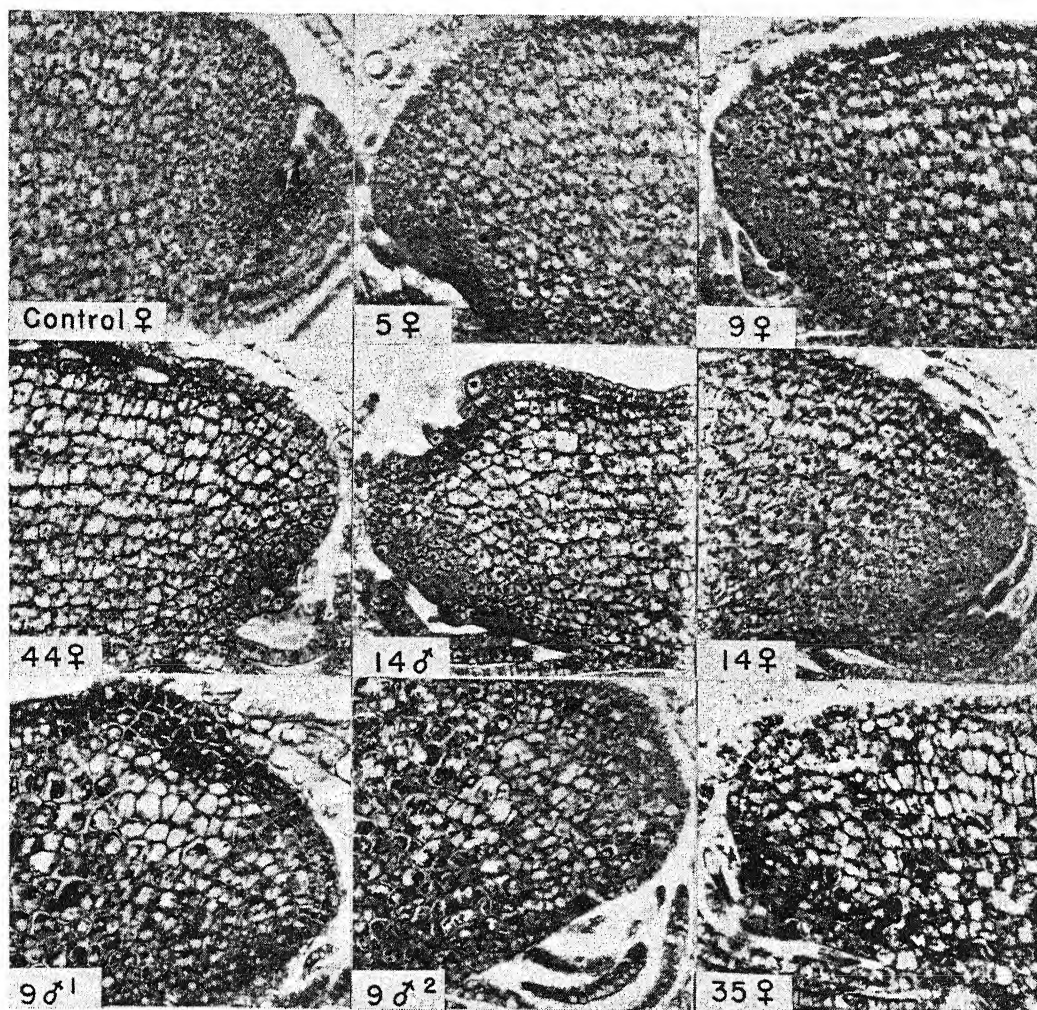


FIG. 5.—Vertical longitudinal sections through apical notch of *M. polymorpha*. Control is growing as stock plant on complete nutrient solution. Numbers indicate position in triangles. Apical cells show most clearly in 44 ♀, 14 ♂, 14 ♀, and 35 ♀.

ened tips showed only advanced stages of terminal degeneration, with no recognizable apical cell or even apical notch area. All sections shown in figure 5 were from tips which possessed no external evidence of necrosis, such as discoloration, and no collapse.

First evidences of unhealthy conditions found in longitudinal sections through the tip of a calcium-deficient plant are the larger vacuoles in the cells of the maturing region, the darker-staining nuclear zones, and the smaller number of chloroplasts. These conditions are shown in figure 5, position 9 ♀ (and to a lesser degree position 5 ♀), even though plants in the former position possessed no outward evidences of unhealthy development, while many plants in the latter culture had blackened tips. REED (10) describes the premature vacuolization and polarization of meristematic cells of the stem tips of apricot and peach trees and the accumulation of phenolic materials in maturing cells when zinc is deficient, but subsequent necrosis when this element is lacking or deficient is not reported.

Associated with the internal symptoms just noted, the meristematic zone is limited to a few concentric layers of cells derived from the apical cell, all containing a relatively large proportion of protoplasm and small vacuoles (fig. 5, 9 ♀ and 5 ♀). When such abrupt zonation becomes especially evident, the apical cell is easily distinguishable (fig. 5, 44 ♀ and 14 ♂). Meristematic activity continues in the dorsal region, where air chambers develop (fig. 5, 44 ♀). Gemmae-cup differentiation also continues (fig. 5, 14 ♂), even when cells below the single floor layer of the cup are mature—as shown by large cell size and extreme vacuolization. The ventral segment of the apical cell in position 44 ♀ (fig. 5) is in the process of transverse division, but the spindle is not clear because of the reduction in size of this figure.

Following the period of limited meristematic activity with concomitant preponderance of mature cells, necrosis in the dorsal areas (near the tip of the plant) occurs (fig. 5, 14 ♀). At times this zone of dead cells involves an embryonic gemmae cup (fig. 5, 9 ♂¹). The breakdown of dorsal tissues is in contrast to the longevity and persistence of the small ventral cells which surround the bases of smooth rhizoids and are the source of regenerated thalli (15).

Most tips in which necrosis is beginning possess apical cells which apparently are still functioning. Sections of a single tip in position 9 (fig. 5, 9 ♂¹ and 9 ♂²) show that dichotomy is still in progress, since different apical cells are present in these sections, which are located on one microscope slide. One section apparently has a living apical cell, while a few microns farther on (fig. 5, 9 ♂²) the other half of the dichotomy possesses a necrotic apical cell region and a zone of living cells forming a V just posterior to the apex. As in position 35 ♀ (fig. 5), all cells of the tip eventually die.

The next event usually is collapse of the cells of the tip, accentuated or even

initiated by decay. Cells in the interior of the older portions of the thallus collapse (15, figs. 11, 12). Regeneration of plants from the small cells surrounding the base of smooth rhizoids located a few millimeters posterior to the tip has been described previously (15).

The rank of male and female plants in the upper two triangles of figure 3 indicates that, despite the unhealthy internal condition of most of the tips in the $\frac{1}{5}$ Ca row, accumulation of dry weight was better than average and in some instances nearly maximum for this experiment. Until the tips began to blacken, the gross appearance of these plants was among the best in the cation triangle.

VEGETATIVE CHARACTERS CORRELATED WITH SEX

In experiments 8 and 9 (15), where antheridial and archegonial plants were not grown simultaneously, the number of gemmae cups on the female plants was considerably less than on the male plants (568 on ♀, 2300 on ♂; a ratio of 1:4), but no significance was expressed since the data were insufficient to warrant a conclusion. By growing both sexes simultaneously under comparable conditions in the experiment under discussion, the earlier results were confirmed (946 gemmae cups on female, 6268 on male plants; a ratio of 1:6.6) and a rough correlation between sex and gemmae-cup production was established. Total gemmae-cup counts for each culture, sex, and triangle are given in figure 6.

Other factors being equal, decrease of the phosphate supply tends to favor greater gemmae-cup production. When all phosphates are absent, however, the number of cups on male plants tends to decrease while on female plants the number increases. Cup ratios are average when phosphates are present in as little as $\frac{1}{9}$ proportion (0.47 millimols per liter); but when phosphates are omitted the ratios are much lower, so that a male plant cannot be distinguished from a female on this basis. As shown in figure 6, decreased nitrate supply results in the production of fewer gemmae cups. Maximum number of cups seems to depend, therefore, largely upon a high nitrate level and a very low phosphate supply. When nitrate supply is high and all common ions are present in the nutrient solution, the sex of vigorously growing cuttings of *M. polymorpha* may be determined reasonably accurately by observing the thallus width approximately 1 cm. back of the apical notch and of the wings. Male plants are narrow when compared with female plants.

The upper surface of vigorously growing female plants is smoother and reflects light more readily than similarly cultured male plants. Whether this appearance is dependent upon differences in cuticle, size of air chambers, curvature of air-chamber roof, relative protrusion of chimney cells of the pore, or upon a combination of these factors is not yet determined.

In general the margins of the male plant are more undulating and tend to curve downward abruptly, especially if infested with blue-green algae or growing in a

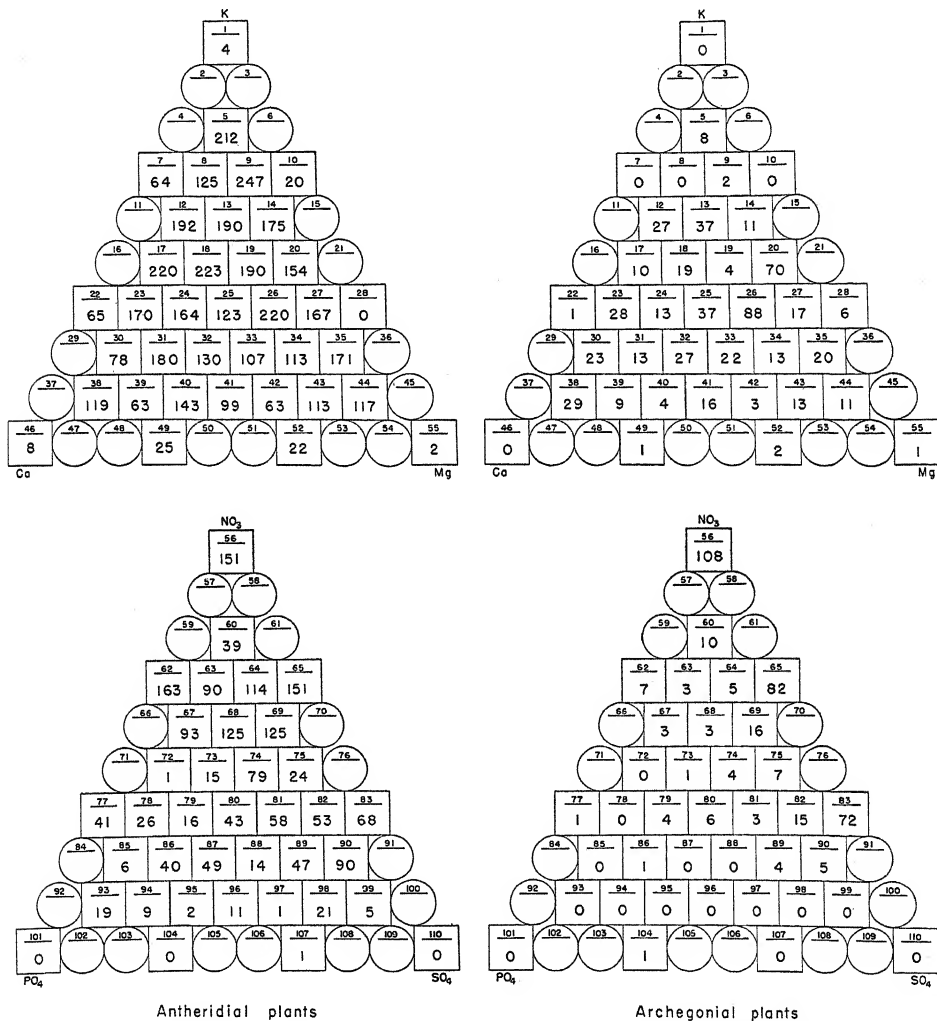


FIG. 6.—Number of gemmae cups on six plants of each culture. Counts on male plants in two triangles at left, female at right. Counts include cups present on original cutting. Ratio of cups on all male plants to those on female is 6.6:1. In anion triangle greatest cup number is associated with decreasing amounts of PO₄ in nutrient solution. Omission of PO₄ results in very slight differences in number of cups on male and female plants. Larger numbers of gemmae cups and higher nitrate supply correlated positively.

medium with a high pH. Often the marginal row of ventral scales tends to curve out and up, forming a wavy white margin in the male plants. Under these conditions, the smaller number of gemmae cups, the broader thallus tip, and the plane

surface of the female plants, known as culture B, serve to distinguish it from culture A, the male plants. These distinctions have definite taxonomic value in the clones just mentioned. Even with a low calcium supply (fig. 4), the distinction between culture A (bottom) and culture B (top) is possible on the basis of gemmae-cup number and gross appearance. Cultures of *M. polymorpha* plants from widely scattered localities in the United States are now being studied to determine the validity of these observations on other clones and to determine a possible correlation between sex and the relative number of gemmae cups produced by any particular culture. If a positive correlation is found, indicating possible sex linkage, further inquiry into the mechanisms of gemmae-cup initiation and factors influencing their development would be highly desirable. If maleness and the presence of large numbers of gemmae cups are not associated in other clones, an extensive field of genetic investigation would be opened. Similarity in number of gemmae cups on both male and female gametophytes when phosphates are absent indicates a need for study of the possible relation of this ion to the factors concerned in the expression of sexual characters.

GAMETANGIOPHORE PRODUCTION

On the 888 plants grown during the present study, only eleven antheridiophores and three archegoniophores differentiated. This response is to be expected, since light intensity decreased during the term of the experiment (September–October).

Summary

1. As in previous experiments with *Marchantia polymorpha*, the omission of K, Ca, NO_3 , or PO_4 ions results in characteristic differences in the gross appearances of the plants. The lack of K produces plants with tan-colored bases and slightly narrower tips. Absence of Ca results in almost immediate death of the growing tips. Deficiency of NO_3 and PO_4 is indicated by reddening of scales, of rhizoids, and of lower epidermis. Plants lacking the former ion become light green, possess few gemmae cups, and fork infrequently; but plants growing on solutions lacking PO_4 soon become dark green, have abundant gemmae cups, and because of frequent dichotomy are rosettes. Deficiencies of Mg and SO_4 are not indicated by any characteristic symptoms.

2. With a concentration of 0.3 millimols of calcium per liter of solution, death and degeneration of the plant apices result. Microscopically an early and rapid vacuolization occurs in enlarged cells near the apical cell. Necrosis first begins in the more nearly mature cells and continues until the entire tip is dead.

3. When grown on glass cloth and supplied with abundant nitrates, plants with many gemmae cups, narrower thalli, and incurved margins are male, belonging to culture A. Plants of culture B (female) have fewer cups, broader thalli, and plane surfaces.

4. On the basis of dry-weight accumulation and total area of plants, optimum vegetative growth is evident in certain areas of each triangle. On this basis, and with consideration of the gross and microscopic aspects of the plants, molar concentrations of the six ions may be suggested as meeting the requirements of *M. polymorpha*: K 0.0012, Ca 0.0007, Mg 0.0014, NO_3 0.0034, PO_4 0.0004, and SO_4 0.0008 mols per liter. In practice, a solution may be made up using the following quantities of a 0.5*M* solution of each of the following salts: KNO_3 1.6 cc., $\text{Ca}(\text{NO}_3)_2$ 1.4 cc., $\text{Mg}(\text{NO}_3)_2$ 1.2 cc., KH_2PO_4 0.8 cc., and MgSO_4 1.6 cc. per liter of solution. Slight variations in the proportions of these salts do not affect the mineral requirements of this plant.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. BAMFORD, RONALD, Changes in root tips of wheat and corn grown in nutrient solutions deficient in calcium. *Bull. Torrey Bot. Club* 58:149-178. 1931.
2. DAY, DOROTHY, Some effects of calcium deficiency on *Pisum sativum*. *Plant Physiol.* 4:493-506. 1929.
3. DE TURK, E. E., Plant nutrient deficiency symptoms. *Physiological basis. Indust. and Eng. Chem.* 33:648-653. 1941.
4. LUTMAN, B. F., Cell size and structure in plants as affected by various inorganic elements. *Vermont Agr. Exp. Sta. Bull.* 383. 1934.
5. McMURTREY, J. E., JR., Relation of calcium and magnesium to the growth and quality of tobacco. *Jour. Amer. Soc. Agron.* 24:707-716. 1932.
6. ———, Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. *Bot. Rev.* 4:183-203. 1938.
7. NIGHTINGALE, G. T., Potassium and calcium in relation to nitrogen metabolism. *BOT. GAZ.* 98:725-734. 1937.
8. NIGHTINGALE, G. T., ADDOMS, RUTH M., ROBBINS, W. R., and SCHERMERHORN, L. G., Effects of calcium deficiency on nitrate absorption and on metabolism in tomato. *Plant Physiol.* 6:605-630. 1931.
9. REED, H. S., The value of certain nutritive elements to the plant cell. *Ann. Bot.* 21:501-543. 1907.
10. ———, Effects of zinc deficiency on cells of vegetative buds. *Amer. Jour. Bot.* 28:10-17. 1941.
11. SCHREINER, OSWALD, and SKINNER, J. J., The triangle system for fertilizer experiments. *Jour. Amer. Soc. Agron.* 10:225-246. 1918.
12. SOROKIN, HELEN, and SOMMER, ANNA L., Changes in the cells and tissues of root tips induced by the absence of calcium. *Amer. Jour. Bot.* 16:23-39. 1929.
13. ———, Effects of calcium deficiency upon the roots of *Pisum sativum*. *Amer. Jour. Bot.* 27:308-318. 1940.
14. TRUE, R. H., The function of calcium in the nutrition of seedlings. *Jour. Amer. Soc. Agron.* 13:91-107. 1921.
15. VOTH, P. D., and HAMNER, K. C., Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. *BOT. GAZ.* 102:169-205. 1940.

EFFECT OF PHOTOPERIOD AND TEMPERATURE ON DEVELOPMENT OF BARLEY

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(WITH TWO FIGURES)

Introduction

The role of artificial light used to extend a natural photoperiod is different in short- and long-day plants in that it prevents flowering in the former and induces it in the latter. These opposite responses to the same stimulus suggest that the basic reactions influenced by supplemental light may be different in the two cases. One source of evidence for or against this assumption would be found in comparison of the intensities of light required to produce these characteristic responses in typical long- and short-day plants. Most of the data available for such comparisons, however, come from experiments dealing with the practical aspects of the use of supplemental light in the control of flowering. The records from most of these are based on the final presence or absence of open flowers or large buds in the experimental material and do not deal with the early stages of floral initiation. Such records are entirely satisfactory for the purpose for which they were intended, but they are not useful for the comparisons here proposed. They show that certain light treatments result in failure of the plants to flower but give no indication of the reason for the failure.

Early examination of the experimental material by dissection methods is required to determine whether the lack of flowers results from failure of flower buds to be initiated or failure of flower primordia to continue development after they have been initiated. For short-day plants, particularly Biloxi soybean (2, 6), such data are available, but this is not the case for long-day plants. The ultimate collection of such data from various typical long-day plants was one of the objectives of undertaking detailed studies with barley.

In addition to being generally regarded as long-day in their responses to photoperiod, several cereals, including barley, are reported to exhibit clear-cut interactions between temperature and photoperiod. In these cereals the effects of temperature treatments applied during germination or seedling stages may express themselves during the subsequent development of the plants or at their maturity. Since the cereals may exhibit after-effects of temperature treatment as well as responses to photoperiod, they are well adapted for varied experiments involving either of these factors or their interactions.

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The purpose of these experiments was to study the influence of different photoperiods upon the development of barley seedlings previously subjected to various temperature treatments. The results presented in this paper were obtained from experiments conducted in the springs and summers of 1940 and 1941.

Literature review

GARNER and ALLARD have reported that long photoperiods hastened the heading of wheat, oats, barley, and rye (3). In the winter cereals, however, PURVIS and GREGORY (7) have stated that flowering was accelerated by an initial treatment with either short photoperiods or low temperature, but they emphasized the fact that, in either case, final treatment with long photoperiods was necessary for the completion of flowering. In spring rye, on the other hand, they found that short photoperiods resulted in the formation of increased numbers of leaves before heads were formed, and that heading was thereby delayed by such treatment. It seems desirable, therefore, to regard these cereals as "long-day" plants, as PURVIS and GREGORY suggest, since the heading of both spring and winter varieties is accelerated by the application of long photoperiods, except during the very early stages of the winter ones, when short photoperiods seem more effective.

Both PURVIS and GREGORY (7) and MCKINNEY and SANDO (5), working with rye and wheat, respectively, have reported that the number of leaves formed before spikelet primordia were differentiated was influenced by the temperature during germination and the photoperiod immediately following. Long photoperiod was correlated with lower numbers of leaves and short photoperiod with higher numbers. In spring rye, however, PURVIS and GREGORY found that leaf number was not influenced by germination temperature, while MCKINNEY and SANDO, working with spring wheat, obtained opposite results. The response of spring barley to this factor was not discussed by either group of workers.

The development of the barley spike has been well described and illustrated by LERMER and HOLZNER (4) and by BONNETT (1). BONNETT reported that four true leaves were differentiated in the ungerminated seed, the fourth and youngest consisting of a transverse ridge near the growing point of the embryo. BONNETT's plants developed a total of approximately twelve true leaves from the main axis, before the primordia of spikelets were initiated. This was a somewhat higher number than was produced by the plants in the present experiments, the difference undoubtedly being related to the different environmental conditions of the two experiments.

Plan of experiment

Two varieties of barley were selected, one a strictly winter type and the other a semi-winter type. The former, Kentucky no. 1, C.I. no. 6050, is reported not to head unless it receives a period of low temperature during its early development,

while the latter, Wintex C.I. no. 6127, is known to require little or no low temperature. In these experiments the Kentucky plants failed to head in any of the experimental lots but the Wintex plants headed under all treatments. This report, therefore, deals exclusively with the results obtained from Wintex.

All the plants were started in rooms where they were grown for various numbers of days under constant conditions of light intensity, temperature, and photoperiod. While the plants were in the rooms they were all grown at 65° F. and on a daily photoperiod of 16 hours. The light source was a carbon arc supplemented with about 160 foot-candles of Mazda light. The total intensity at the surface of the leaves was approximately 2000 foot-candles. Beginning May 7, 1940, plantings of both varieties were made at four successive 5-day intervals. Thus on the twentieth day after the first planting, seedlings 5, 10, 15, and 20 days old were available. Differential temperature treatments were started at this time. Three temperatures, 35°, 45°, and 65° F., were provided in separate control rooms. The seedlings of each planting date were divided into three equal lots, and one lot was placed in each room.

While the plants were receiving differential temperature treatment the photoperiod was continued at 16 hours daily, as it had been during their earlier growth, and the quality, duration, and intensity of the light were the same over all lots. Although an attempt was made to hold the temperature of the coldest room at 35° F. continuously, this was not possible during all of each photoperiod because of considerable heat liberated by the lights. During the dark periods the temperature was held at about 35° ± 2° F. and during the photoperiods it was always less than 45° F. Temperatures of the other two rooms did not deviate more than ± 2° from 45° and 65° F., respectively.

The temperature treatments were continued one week; then the plants were transferred to an outdoor area where facilities were available for control of photoperiod. The plants of each treatment were divided into four groups, and those of each group were transplanted in duplicate lots of five to separate photoperiods. The plants were placed in boxes of soil 3½ feet long, 1 foot wide, and 1 foot deep, with two rows of five plants in each box. The boxes were mounted on trucks to permit movement into and out of darkhouses to control length of photoperiod. At the time of transplanting, the various lots were 12, 17, 22, and 27 days old.

Photoperiods of 12, 16, 20 hours, and continuous light were employed. All trucks were removed from the houses at 6:00 A.M. daily and returned at 6:00 P.M. Those on 12-hour photoperiod were placed in darkness at 6:00 P.M. but all others were subjected to Mazda light inside their respective houses until the desired length of photoperiod had been attained. In the 16-hour chamber the lights went out at 10:00 P.M.; in the 20-hour chamber they went out at 2:00 A.M.; while in the continuous-light room the lights were on from 6:00 P.M. to 6:00 A.M. All lots

thus received the same amount of natural light each day but differed in the amount of artificial light received. Since the intensity of artificial light, 30-40 foot-candles, was very low in comparison with that of natural light, the total amounts of radiant energy received by plants on long and short photoperiods did not differ greatly.

Following transplanting, daily records were made of the number of days from planting to awn appearance on the individual plants. These data were recorded when the first visible awn emerged from the boot. In the statistical analysis of the data these records were grouped into totals per row. The results are reported in the tables as means per plant.

The plants remained under the differential photoperiods until the grain on the 16-, 20-, and 24-hour photoperiods was mature. At this time all the plants were harvested, even though those on 12-hour photoperiod were still green and making vegetative growth. The roots of the plants were discarded and the entire tops dried at 80° C. The dry weight of the tops per lot of five plants was first determined. The heads were then threshed and the yield of seed determined.

The treatments in each of the four dark chambers available in the outside area were arranged in duplicate series and randomized within groups. In the analyses of the results these two groups were treated as duplicates, although strictly speaking the photoperiodic conditions were not duplicated. To have duplicated each condition would have necessitated a reduction in the number of kinds of photoperiodic treatments that could have been conducted. It was felt more desirable in this first experiment to retain the four photoperiodic conditions and to assume that differences between dark chambers were slight rather than to provide a measure of this factor through duplication of half as many kinds of treatments. The results were subjected to variance analysis and, in the discussion, where significance is attached to any differences reported, the odds were 99 to 1 or higher.

The variables studied in the experiment included four planting dates, three temperature treatments of 1-week duration during the seedling stage, four conditions of photoperiod during the later development of the plants, and duplicate lots throughout. This represents 48 kinds of duplicated treatments, or 96 separate lots. Each lot contained five plants, so the entire experiment required 480 plants of each variety.

So many plants were required that space in the control rooms was not available to produce extra plants for dissection during their early stages prior to and following the temperature treatments. To determine the approximate condition of the seedlings at various ages, another planting was made the following year and used exclusively for that purpose and for photographic records. The conditions of light intensity, temperature, and photoperiod in the rooms were made identical with those in the first experiment. This second experiment was planted on May 6, 11, 16, and 21, 1941, one day earlier than the 1940 experiment. The same time of

year was selected so that plants not needed for dissection could be transferred outside under conditions comparable with those of the previous year. The plants grown outside in 1941, however, were transplanted to 6-inch pots instead of to boxes of soil, to permit regrouping for photographic purposes. All photographs shown are from the 1941 experiment.

After the plants were dissected they were preserved in a mixture of formalin-alcohol-acetic acid until they could be photographed. Before photographing they were removed from the preservative, washed, and then placed in an aqueous solution of methyl green. As soon as the primordia had acquired a deep blue color they were ready for photographing. The material did not overstain objectionably if it was left in the stain for several days.

The stained material was photographed under about half an inch of water, where it was held in position by a needle inserted in the base of the stem. The needle was mounted in a universal joint in such a way that the material could be readily rotated in any plane. The whole assembly could be raised and lowered with a rack and pinion. Most of the photographs were made at a magnification of 12 diameters with a 32-mm. micro-Tessar lens mounted in a 5×7 camera. The primordia were illuminated by two small beams of light directed obliquely downward at them in such a way that excess light did not illuminate the black background at the bottom of the tank. This method of handling the material makes possible the collection of many samples in a short time, the photographic records being completed as time permits.

The data include dissection records made at the beginning and end of the temperature treatments, mean number of days from planting to awn emergence, dry weights of mature plants and of seed per plant, and mean number of seeds per plant.

Results

Under the conditions of these experiments the seedlings of all lots emerged from the soil 4 days after planting. The stage of development attained by representative plants of each lot when 5, 10, 15, and 20 days old is illustrated in the first column of figure 1. Beginning at this time the plants were subjected to three different temperatures for one week. At the expiration of the week representative plants were again photographed. Those of each planting that had received 7 days at 65° F. are shown in the second column of figure 1; the ones that received 45° F., in the third column; and those that received 35° F. are shown in the last column. Corresponding lots of plants held at the two lowest temperatures did not differ greatly at the end of a week of treatment. Plants of both lots, however, were definitely smaller than those of the lots that had been continued at 65° F. during this time.

At the time the temperature treatments were started the oldest seedlings had formed only five visible leaves. The formation of new structures at the growing points of the main axes of these seedlings, however, was already complete. The average number of nodes in the main axes of the eight plants dissected was 32.5 (table 1), and in the plants dissected a week later there were no additional structures present.

Plants of the three later plantings had not yet completed the differentiation of new structures in their main axes when the temperature treatments were started. During the week of temperature treatments all lots continued differentiation, but the rate was much greater in the older than in the younger seedlings. By the time the temperature treatments were completed all the plants of the second planting had practically completed the differentiation of new structures in their main axes.

TABLE 1
EFFECT OF LOW TEMPERATURE ON PRODUCTION OF NODES IN
WINTEX BARLEY SEEDLINGS OF VARIOUS AGES.
AVERAGES BASED ON 8 PLANTS

AGE (IN DAYS) AT BEGINNING OF TEMPERATURE TREATMENT	TOTAL NODES AT BEGINNING OF TEM- PERATURE TREATMENT	TOTAL NODES AFTER 7 DAYS' TREATMENT		
		65° F.	45° F.	35° F.
20.....	32.5	31.4	32.1	32.4
15.....	23.1	32.3	31.8	30.6
10.....	9.1	31.9	15.3	12.6
5.....	5.0	14.7	7.4	6.9

The plants of the 65° lot of the third planting were also nearly complete at this time, but the two low-temperature lots of this planting and all lots of the last planting had fifteen or fewer nodes per plant when transplanted.

The beginning of spikelet formation in plants grown continuously at 65° F. occurred between the tenth and twelfth days (fig. 1). On the tenth day the collar-like rudimentary leaves found at the base of the spike were just forming. On the twelfth day spikelet primordia were conspicuous at six or more nodes of the rachis above these leaves. The rate of development of the young spike proceeded rapidly under the conditions of these experiments. Stages of development of representative plants grown continuously at 65° and of the growing points dissected from these are shown in the first two columns of figure 1. In the first column the plants with their growing points were 5, 10, 15, and 20 days old from top to bottom, and in the second column they were 12, 17, 22, and 27 days old.

The most obvious effect of the two low-temperature treatments was to delay development of the spikes. In the younger plantings the low temperature delayed

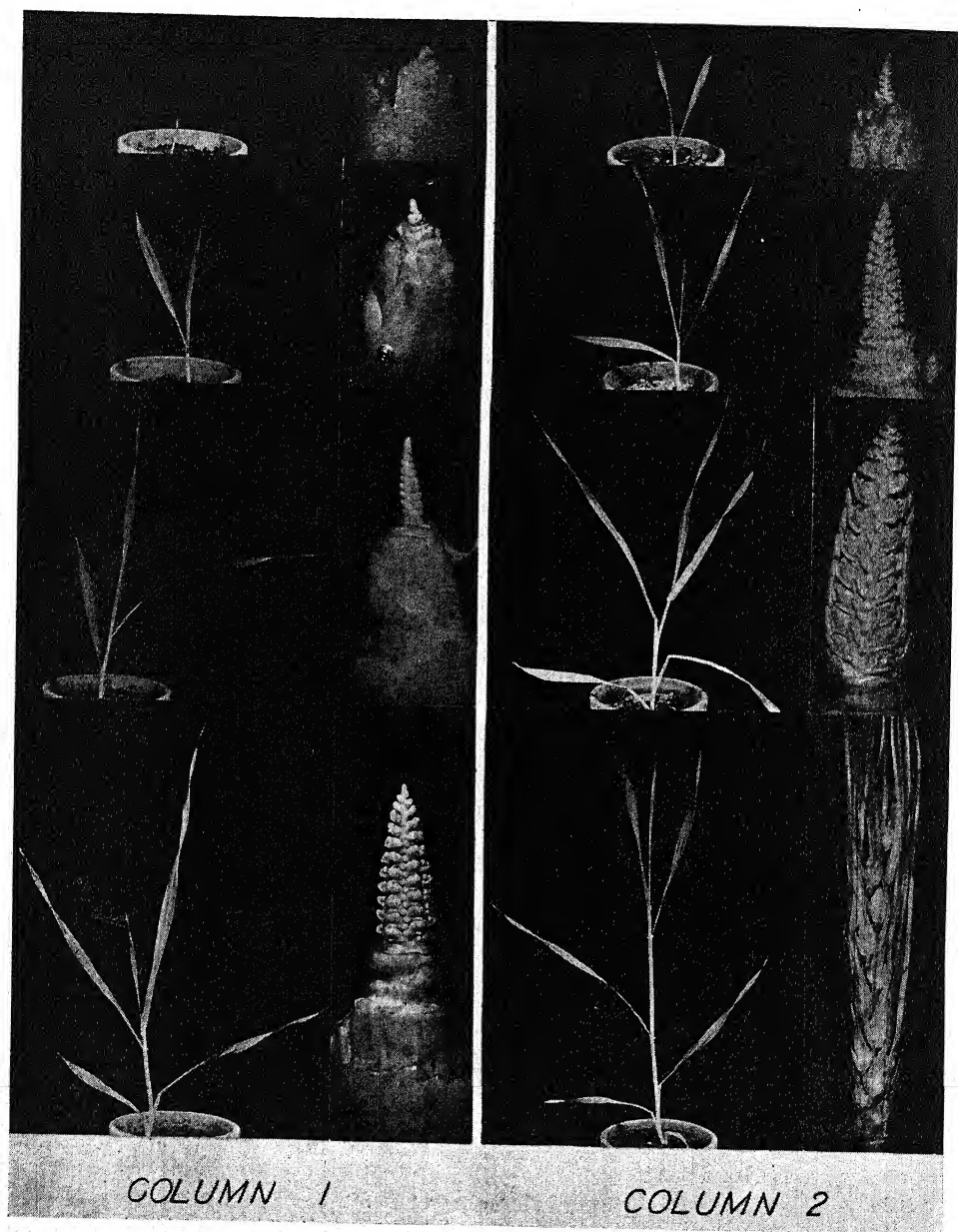


FIG. 1.—Seedlings from four successive plantings of Wintex barley grown on 16-hour photoperiods and under several conditions of temperature; last planting in top row and first in bottom row. Enlargement of terminal of main axis shown at right of seedling from which it was dissected. Seedlings of first column 5, 10, 15, and 20 days old, respectively, from top to bottom. Seedlings of second, third, and fourth columns all one week older than corresponding seedlings of first column. All seedlings grown at 65° F. at all times except those of columns three and four, which were transferred to 45° and 35° F., respectively, one week immediately prior to photographing. Approximate magnifications: all seedlings $\times 4\frac{1}{2}$; enlarged head in bottom row of column two $\times 4\frac{1}{2}$; all other terminals $\times 9\frac{1}{2}$.

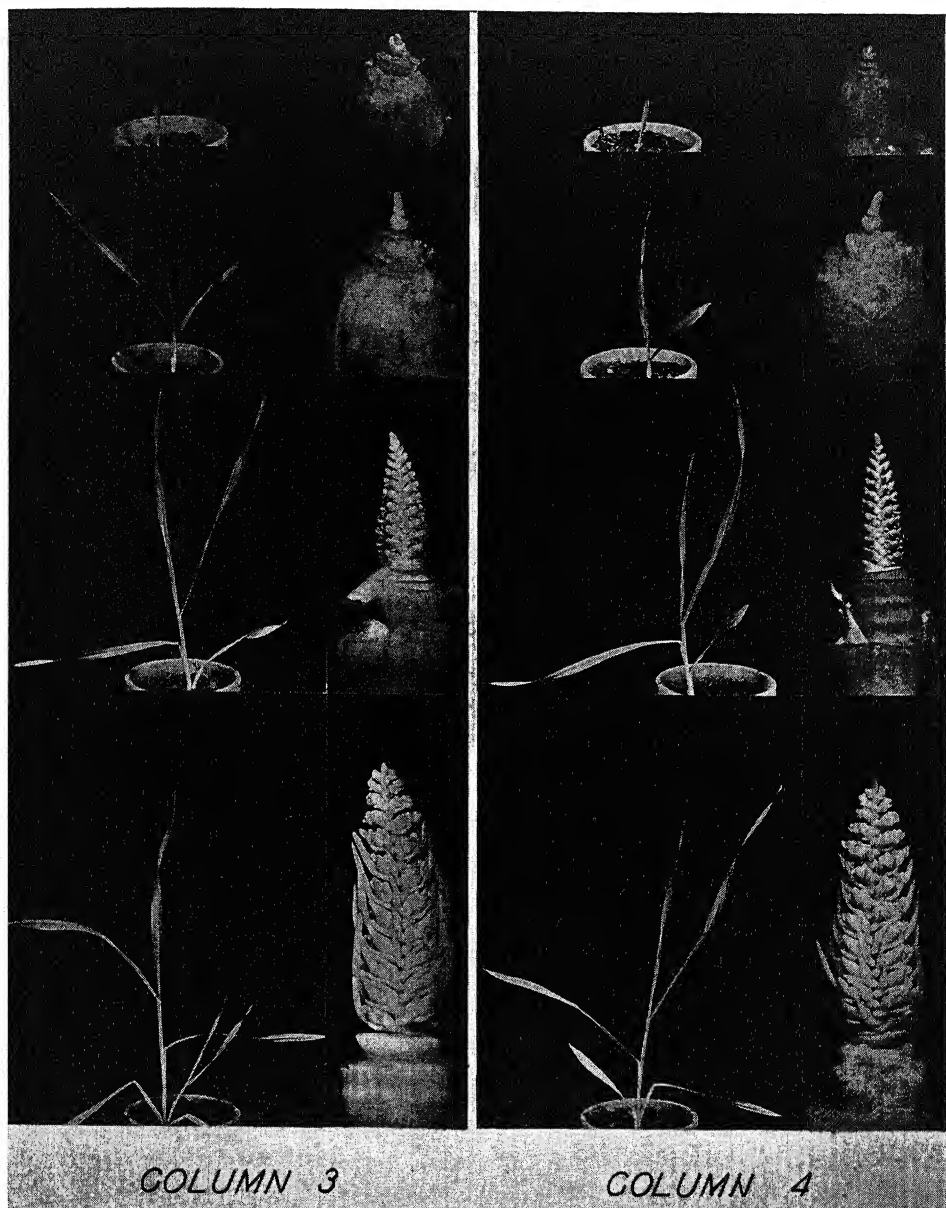


FIG. 1.—*Continued*

development of structures already present and retarded the differentiation of new ones. In the oldest planting the effect was only upon the rate of growth of primordia already established, because differentiation of new organs was complete before the low temperatures were applied. The amount of delay appeared approximately the same under both low temperature conditions (fig. 1, columns 3 and 4), but dissection data (table 1) indicate that it was actually somewhat greater in the 35° than in the 45° F. lot.

After transplanting to the boxes in the outside area, daily records were made on the appearance of awns on the main shoots of the individual plants, and photographs were made at frequent intervals. The stage of development attained by representative plants of the various lots on June 24, 22 days after transplanting, is shown in figure 2. At this time awns were visible on the plants of all treatments of the first planting and in some the heads had fully emerged from the leaf sheath. In the second planting awns were visible in all lots except the two low-temperature ones growing on 12-hour photoperiods. In the third planting no awns were visible in any of the 35° or 45° lots, but they had emerged in the 16-, 20-, and 24-hour lots of the 65° F. group. In the fourth planting at this time awns were visible only on the plants grown at 65° on 20- and 24-hour photoperiods.

The mean number of days required from planting to awn emergence varied with the photoperiod, age of plants, and temperature treatment (table 2). Of these factors, photoperiod produced the greatest effects. Awns appeared first on the plants receiving 24-hour photoperiod, and these were closely followed by those receiving 20- and 16-hour photoperiods. Awn appearance was delayed most in the plants receiving 12-hour photoperiods, particularly in those of the last two plantings. The plants from these two plantings, with the exception of the 65° F. lot of the third planting, had not completed differentiation of all spikelet primordia at the time of transplanting. The influence of short photoperiod on these plants could therefore affect both their further differentiation and their subsequent development and growth. Since differentiation of spikelets was complete in the plants of the first and second plantings and in the 65° F. lot of the third planting, short photoperiod affected the time of awn appearance only through its effect upon the growth and elongation of these previously differentiated structures. The character of growth of the plants receiving 12-hour photoperiods differed from that of the plants receiving 16-, 20-, or 24-hour photoperiods in that none of the elongating shoots ever grew completely erect and most of them were extremely prostrate. These plants formed many more tillers than those on the longer photoperiods, and although they produced spike primordia most of these tillers failed to elongate.

Awns emerged later in all plants that had received a week of low temperature during the seedling stage than in plants of similar lots grown continuously at

65° F. The effects of low temperature treatments on the time of awn appearance were correlated with the influence of these treatments in retarding the differentiation and development of the spikelet primordia previous to transplanting (fig. 1). When seedlings were transplanted to various long photoperiods these early-induced differences did not result in great variations in the date of awn emergence, but when they were transplanted to 12-hour photoperiod the date of awn appearance was delayed. This delay was greater the later the planting.

TABLE 2
EFFECT OF VARIOUS ENVIRONMENTAL FACTORS ON MEAN NUMBER OF DAYS FROM
PLANTING TO APPEARANCE OF AWNS IN WINTEX BARLEY.
AVERAGES BASED ON 10 PLANTS

AGE (IN DAYS) OF PLANTS WHEN DIFFERENTIAL TEMPERATURES WERE APPLIED (GROWN AT 65° F. ON 16-HOUR PHOTOPERIOD)	TEMPERATURE APPLIED FOR 7 DAYS JUST PRIOR TO TRANSPLANTING (° F.)	AGE OF PLANTS WHEN TRANS- PLANTED TO DIFFERENTIAL PHOTOPERIODS AND NATURAL TEMPERATURES	DAYS FROM PLANTING TO AWN EMERGENCE UNDER DIFFERENTIAL PHOTOPERIODS			
			12-HOUR	16-HOUR	20-HOUR	24-HOUR
20.....	65	27	35.1	35.2	35.1	35.0
15.....	65	22	36.7	32.8	31.8	31.9
10.....	65	17	41.6	31.8	31.0	31.0
5.....	65	12	46.8	32.0	30.6	30.2
20.....	45	27	39.2	36.6	36.1	35.5
15.....	45	22	47.1	37.6	36.2	36.1
10.....	45	17	57.7	36.9	35.5	35.2
5.....	45	12	64.9	36.3	33.5	32.4
20.....	35	27	39.9	36.4	36.4	36.2
15.....	35	22	45.5	36.8	36.0	36.0
10.....	35	17	56.5	38.0	35.7	35.8
5.....	35	12	78.7	37.0	33.7	33.4

The actual dates of awn appearance naturally varied from one planting to another. When the time of awn appearance was calculated from the time of planting, however, these differences were not great for any except the 12-hour photoperiod. The younger the plant when treatments were applied, the greater the retardation resulting from 12-hour photoperiod.

The mean dry weights of the plants were correlated with the photoperiods to which they were subjected after transplanting. The plants grown on 12-hour photoperiod were much heavier than those of any of the other lots (table 3). These plants produced no grain, in contrast to those grown on 16-, 20-, and 24-hour photoperiods. Their additional weight was due to the greatly increased leaf area resulting from the continued production of tillers throughout the entire growing season. Of the groups that produced grain, the 16-hour photoperiod was the most favorable for production of dry weight and the 24-hour photoperiod the least fa-

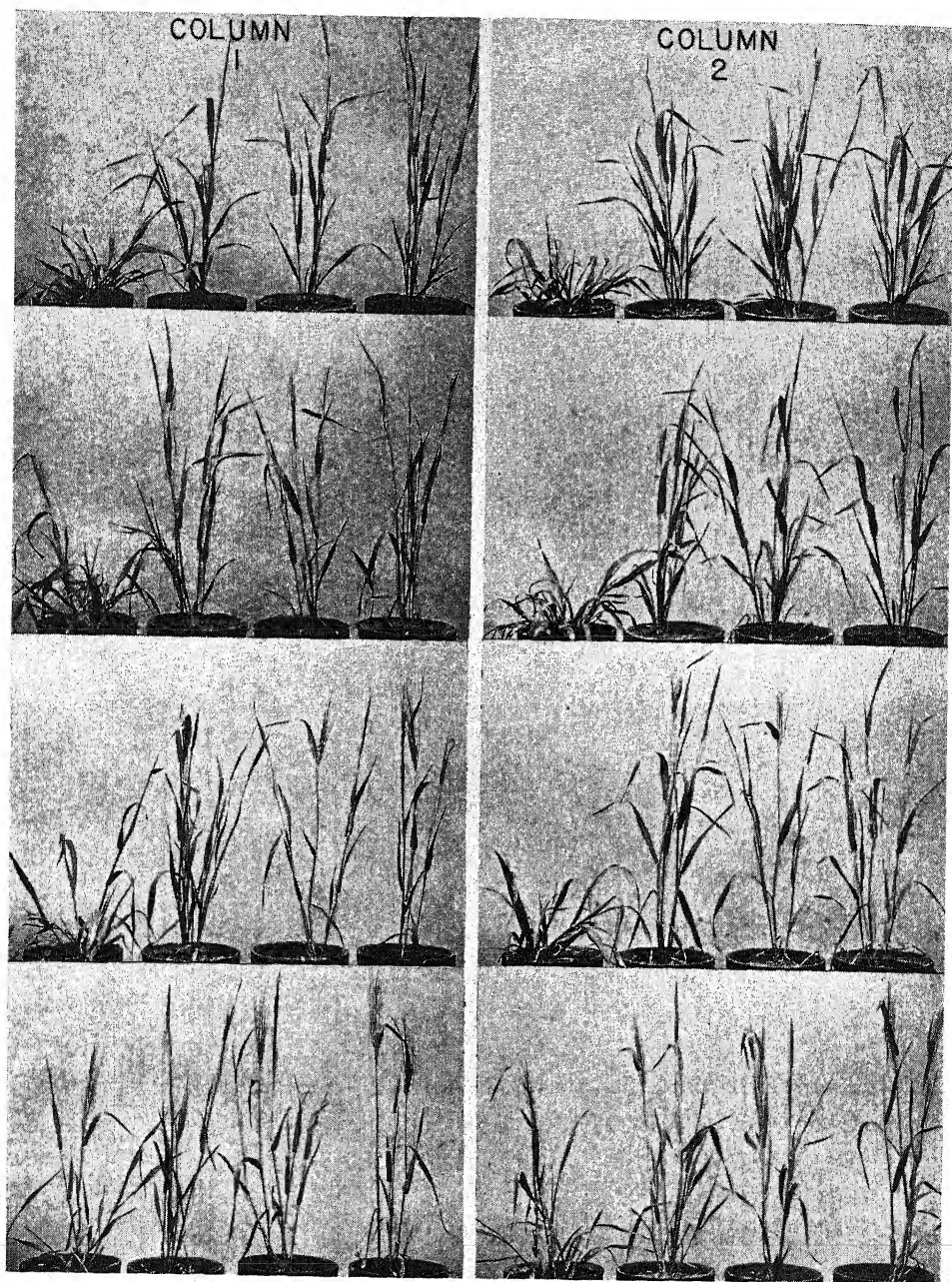


FIG. 2.—Wintex barley plants grown under three conditions of temperature and 16-hour photoperiod before transplanting, then shifted outside to prevailing temperatures and four different photoperiods. Plants from left to right in each group of four plants received 12-, 16-, 20-, and 24-hour photoperiods, respectively, after transplanting. Top row planted May 21, second row May 16, third row May 11, and fourth row May 6, 1941. All transplanted June 2. During week preceding transplanting, plants of first column received temperature of 65° F., those of second column 45° F., and those of third column 35° F. At all other times prior to transplanting all seedlings received 65° F. Photographed June 24, 1941.

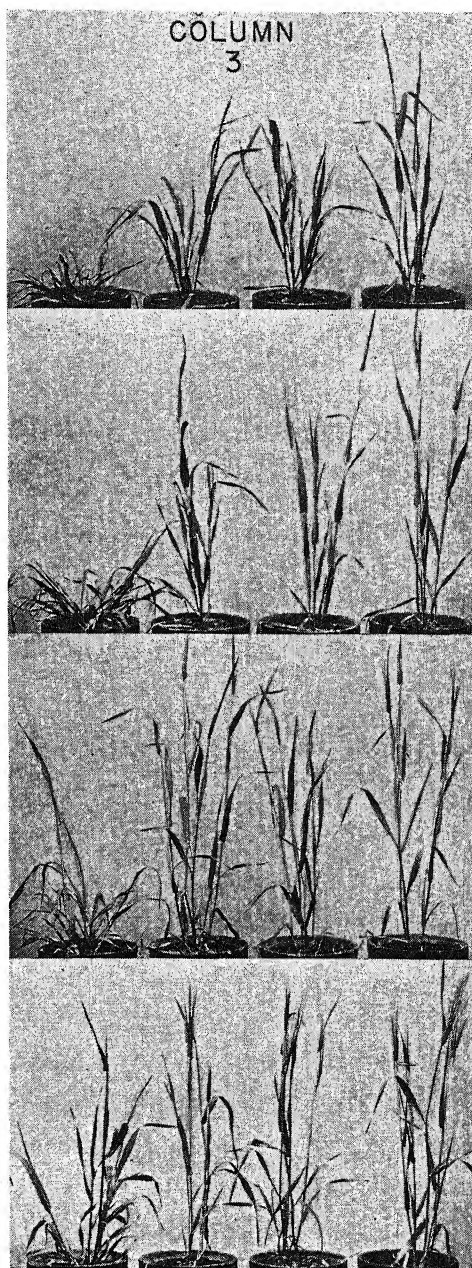


FIG. 2.—Continued

vorable. The differential temperature treatments applied before transplanting caused no significant differences in the accumulation of dry weight. The age of plants was not a factor in this respect since the plants were not harvested until the grain was mature.

TABLE 3
EFFECT OF VARIOUS ENVIRONMENTAL FACTORS ON MEAN DRY
WEIGHT PER PLANT IN WINTEX BARLEY. AVERAGES
BASED ON 10 PLANTS

AGE (IN DAYS) OF PLANTS WHEN DIFFERENTIAL TEMPERATURES WERE APPLIED (GROWN AT 65° F. ON 16-HOUR PHOTOPERIOD)	TEMPERATURE APPLIED FOR 7 DAYS JUST PRIOR TO TRANS- PLANTING (° F.)	MEAN DRY WEIGHT IN GRAMS PER PLANT OF LOTS UNDER DIFFERENTIAL PHOTOPERIODS			
		12-HOUR	16-HOUR	20-HOUR	24-HOUR
20.....	65	9.4	3.7	4.1	3.4
15.....	65	10.2	6.3	4.1	4.0
10.....	65	12.4	6.1	6.8	5.7
5.....	65	18.2	6.0	5.5	4.5
20.....	45	11.2	3.7	4.7	4.6
15.....	45	12.8	6.3	5.8	5.1
10.....	45	15.2	6.5	5.6	4.8
5.....	45	13.9	6.3	5.9	4.1
20.....	35	9.7	4.1	4.5	3.5
15.....	35	10.5	5.0	4.0	3.4
10.....	35	11.9	5.2	4.3	3.5
5.....	35	12.5	5.6	4.3	3.5

TABLE 4
EFFECT OF PHOTOPERIOD WHEN APPLIED TO PLANTS OF VARIOUS
AGES ON MEAN NUMBER OF SEEDS PER PLANT OF WINTEX
BARLEY. AVERAGES BASED ON 10 PLANTS

PHOTOPERIOD	MEAN NO. OF SEEDS PER PLANT FROM LOTS TRANS- PLANTED TO DIFFERENTIAL PHOTOPERIODS			
	12 DAYS	17 DAYS	22 DAYS	27 DAYS
16-hour.....	71	60	58	36
20-hour.....	50	52	41	41
24-hour.....	31	38	29	33

The number of seeds per plant was influenced by the length of photoperiod received after transplanting and by the age at time of transplanting, but not by the temperature treatment received prior to transplanting (table 4). The plants receiving 16-hour photoperiods produced the most grain while those receiving 24-

hour photoperiods produced the least; and the younger the plant at the time of transplanting the greater the yield. This fact was also true for the weight of grain per plant. Since these data show the same trends as those for number of seeds per plant, they are not presented.

Discussion

In barley and other cereals, differentiation of many of the structures found in the mature plant occurs during the first few weeks of seedling development. The rate of differentiation of these structures and the kind of structures found are both influenced by various environmental factors, including photoperiod and temperature. Even slight variations in length of photoperiod or germination temperature may result in the formation of different numbers of leaves before the initiation of spikes occurs. Differences in temperature also result in different rates of development. For these reasons different lots of plants that are not grown under controlled conditions seldom attain the same development at any given age.

In these experiments the conditions of growth prior to the time of transplanting were controlled and therefore reproducible at any time. The plants made vigorous growth and formed primordia of spikelets early in their development. The seedlings were all grown on a 16-hour photoperiod, and those held continuously at 65° F. completed the differentiation of all the nodes of the main axis 17 days after planting. The first primordia of spikelets were formed at the seventh or eighth nodes in nearly all plants. Since three or four of these nodes were present in the ungerminated seed, and one or two additional ones were formed before the seedlings emerged from the soil, it is evident that conditions favoring early spike formation approached the optimum.

Wintex barley resembles spring types in that it is capable of heading without the application of temperatures lower than 65° F. at any time during its development. The effects of the low temperatures applied to Wintex barley in these experiments were manifested through retarded growth rates rather than through after-effects of temperature typical of winter varieties. This retardation in growth as seen in the spike primordia (fig. 1) was very evident before the plants were transplanted to different photoperiods. Under each of the three lengths of photoperiod favorable to early heading these differences induced by low temperature were much less conspicuous by the time of awn emergence (table 2) than they appeared to be at the time of transplanting (fig. 1). Under a 12-hour photoperiod, which was less favorable to early heading, the retardations visible in the seedlings at the time of transplanting became still greater during the later development of the plants.

The awns of plants receiving 12-hour photoperiods appeared later than those of plants subjected to the other three photoperiods. This difference was slight in

the first planting but progressively greater in the later ones. This is to be expected, since the 12-hour photoperiod was applied to the late-planted seedlings earlier in their development, at a time when it could affect their differentiation as well as their growth. It is in this way that the chief influence of low temperature was expressed: it retarded the development of the seedlings so that when they were transferred to short photoperiods they were in an earlier stage of differentiation than others of the same age that had received no low temperature. Short photoperiod was therefore applied to them when they were in still earlier stages of differentiation; and its retarding influence upon awn emergence, acting upon the plants through a longer period of their development, served further to increase the original difference caused by the temperature treatment.

The differences resulting from photoperiodic treatments applied in these experiments are to be attributed to the influence upon the plants of supplemental light of relatively low intensity. Each photoperiod, regardless of length, contained 12 hours of natural light per day, so the main source of radiant energy was identical in all lots. The differences in length of photoperiod were the result of adding supplemental Mazda light of less than 40 foot-candles. Even when this supplemental light was used 12 hours per day, the total energy supplied was exceedingly small in contrast to that supplied by the 12 hours of natural light each day.

The greatest differences resulting from photoperiodic treatment occurred between the 12- and 16-hour lots. The 16-hour ones headed rather uniformly early in all plantings. The 12-hour lots headed nearly as early in the first planting but required progressively more time in each successively later planting. Although awns eventually appeared on every 12-hour plant in the experiment, none of these plants produced a single fertile seed. On the other hand, the plants of the 16-hour lots were all fertile and the heaviest yields of grain came from this photoperiod. These differences resulted from the presence or absence of approximately 40 foot-candles of Mazda light during 4 hours per day.

From these results it seems probable that still lower intensities might have been equally effective in promoting heading and subsequent maturation of seed. More detailed experiments concerning this point are under way.

Summary

1. Four lots of barley seedlings were grown on 16-hour photoperiod to ages of 5, 10, 15, and 20 days, respectively, at 65° F. Each lot was then divided into three groups and all were grown for another week under the same conditions of light and photoperiod. One group of each lot, however, was continued during the week at 65° F., one was transferred to 45° F., and the third to 35° F. The plants were then removed from the control rooms and transplanted out of doors for final differential photoperiodic treatment.

2. At the beginning and end of the temperature treatments representative plants from each lot were photographed and dissected. The total number of nodes in the main axis of each was determined, and enlarged photographs of the terminals of the main axes were made.

3. Ten plants from each age and temperature group were subjected to 12-, 16-, 20-, and 24-hour photoperiods after they were moved outside and remained on these photoperiods at natural temperatures for the duration of the experiment.

4. Number of days from planting to awn emergence was determined. At maturity the dry weights of the tops and of the grain were also determined, and counts were made of the number of seeds per plant.

5. Delay in awn emergence resulted from 12-hour photoperiod; the younger the seedling at the time of transfer to this photoperiod the greater the delay. Delay in awn emergence resulted from low temperature applied during the seedling stage. The greatest delay occurred when the youngest seedlings were subjected to a week of low temperature and were subsequently grown on 12-hour photoperiods. Typical temperature after-effects were not observed; the influence of low temperature was expressed through its effect on the growth rate while it was being applied.

6. The plants receiving 12-hour photoperiod did not produce a single fertile seed, but they produced more dry weight than the lots receiving longer photoperiods. The 16-, 20-, and 24-hour photoperiods resulted in lighter plants but all were fertile. Among these three lots best yields in number and weight of seed were obtained from plants on 16-hour photoperiod.

7. The differences resulting from the photoperiodic treatments have been attributed to the influence upon the plants of low-intensity supplemental light.

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LITERATURE CITED

1. BONNETT, O. T., The development of the barley spike. *Jour. Agr. Res.* 51:451-457. 1935.
2. BORTHWICK, H. A., and PARKER, M. W., Influence of photoperiods upon the differentiation of meristems and the blossoming of Biloxi soybeans. *BOT. GAZ.* 99:825-839. 1938.
3. GARNER, W. W., and ALLARD, H. A., Further studies in photoperiodism: the response of the plant to relative length of day and night. *Jour. Agr. Res.* 23:871-920. 1923.
4. LERMER, J. K., and HOLZNER, G., Beiträge zur Kenntnis der Gerste. München. 1888.
5. MCKINNEY, H. H., and SANDO, W. J., Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. *Jour. Agr. Res.* 51:621-641. 1935.
6. PARKER, M. W., and BORTHWICK, H. A., Effect of photoperiod on development and metabolism of the Biloxi soybean. *BOT. GAZ.* 100:651-689. 1939.
7. PURVIS, O. N., and GREGORY, F. G., Studies in vernalisation of cereals. I. A comparative study of vernalisation of winter rye by low temperature and by short days. *Ann. Bot. n.s.* 1:569-591. 1937.

EFFECT OF NUTRITION AND AGE UPON RATE OF DEVELOPMENT OF TERMINAL STAMINATE INFLORESCENCES OF *XANTHIUM PENNSYLVANICUM*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 533

AUBREY W. NAYLOR

(WITH THREE FIGURES)

Introduction

Attention has recently been called to the effect of a number of environmental factors on floral initiation in soybean (*Soja max*) and cocklebur (*Xanthium pennsylvanicum*). These studies have been, for the most part, designed to determine the effect of (a) photoinductive cycles longer than 24 hours (16); (b) high and low temperatures during the photoinductive cycles (2); and (c) light intensity previous to, during, and after the photoinductive cycle—on floral initiation without regard to subsequent development. Some data, however, are given concerning the rate of development subsequent to initiation (6, 10, 13, 16). In practically all cases, however, the photoperiods employed were considerably above the critical.

Although some work has been done to determine the effects of different types of environments on the critical day length, few data are available on rate of development subsequent to photoinductive cycles near the critical. Temperature during the dark period, humidity, and light intensity were each found by LONG (10) to be important factors in altering the critical day length of *X. pennsylvanicum*. Also in *Rudbeckia bicolor*, a long-day plant, increase in temperature from 25° to 28.3° C. resulted in decrease in the critical day length (17).

The present work deals with the effect of nutrition and relative age of leaf tissue on the initiation and rate of development of floral primordia subsequent to treatments with photoinductive cycles at the critical and above.

Material and methods

The experiments reported here were performed in the University of Chicago greenhouses during the spring and summer of 1939. Burs of *Xanthium pennsylvanicum* were collected in Chicago during the autumn of 1938 and stored out-of-doors until early in 1939. In February they were dried and kept inside until shortly before use. Then the seeds were removed from the fruit, nicked slightly to rupture the seed coats, and planted. In all cases the seeds were germinated in flats on well-lighted greenhouse benches where supplementary illumination of about 100 foot-candles was supplied from shortly before sunset to 2:00 A.M. by Mazda filament lamps. Young seedlings, after attaining 4-6 inches in height (except in

the nutrition experiment), were transplanted to $3\frac{1}{2}$ -inch clay pots. In the nutrition experiment the seeds were germinated in sand and the plants transferred to small glazed crocks filled with sterile sand, two plants being used in each crock. After transplanting, all plants were maintained on long photoperiods (18–20 hours of light and 6–4 hours of dark) until experimental treatment was begun.

Inasmuch as the soil was rapidly leached by the application of tap water twice daily, it was found necessary to add a complete nutrient solution at frequent intervals to prevent the plants from showing deficiency symptoms. This nutrient also served as the basic solution (table 1) for the nutrition experiment.

TABLE 1
CONCENTRATION OF CONSTITUENTS OF COMPLETE AND
DEFICIENT SOLUTIONS AS APPLIED TO THE PLANTS

SOLUTION	CONSTITUENTS OF SOLUTIONS
Complete nutrient.....	$\text{Ca}(\text{NO}_3)_2$0.006 molar KH_2PO_40.0045 molar MgSO_40.0045 molar $\text{B}(\text{H}_3\text{BO}_3)$0.5 p.p.m. Fe as ferric citrate...1 cc. 0.5% solution/liter MnCl_20.5 p.p.m. CuCl_20.125 p.p.m. ZnCl_20.5 p.p.m.
Minus nitrogen.....	CaCl_2 substituted for $\text{Ca}(\text{NO}_3)_2$
Minus potassium.....	NaH_2PO_4 substituted for KH_2PO_4
Minus phosphorus.....	KCl substituted for KH_2PO_4

Stock plants were dissected at frequent intervals to make certain that all were vegetative. At the time of harvest the terminal inflorescence primordia were arbitrarily classified into seven different stages of development, varying from the vegetative bud to barely submacroscopic inflorescences. This classification is similar to that used by MANN (11).

Procedure and results

EFFECT OF MINERAL NUTRITION AND LENGTH OF DARK PERIOD UPON INITIATION AND DEVELOPMENT OF INFLORESCENCE PRIMORDIA

Previous experiments (7) have shown that under ordinary greenhouse conditions of summer, *X. pennsylvanicum* requires at least $8\frac{1}{2}$ hours of darkness out of 24 before it will initiate floral primordia. Inasmuch as mineral nutrition exerts considerable effect on development subsequent to initiation, this experiment was designed to determine the effect of low nitrogen, low potassium, and low phosphorus on ability to initiate floral primordia and the rate of their subsequent development.

On June 8, seeds were planted in washed quartz sand which was watered periodically with nutrient solution. After germination, when the seedlings were ex-

panding their first leaves (June 23), selections were made for uniformity in vigor and development. Two of these young plants were placed in each small crock and the sand thoroughly watered. Twenty crocks were placed in each of the small

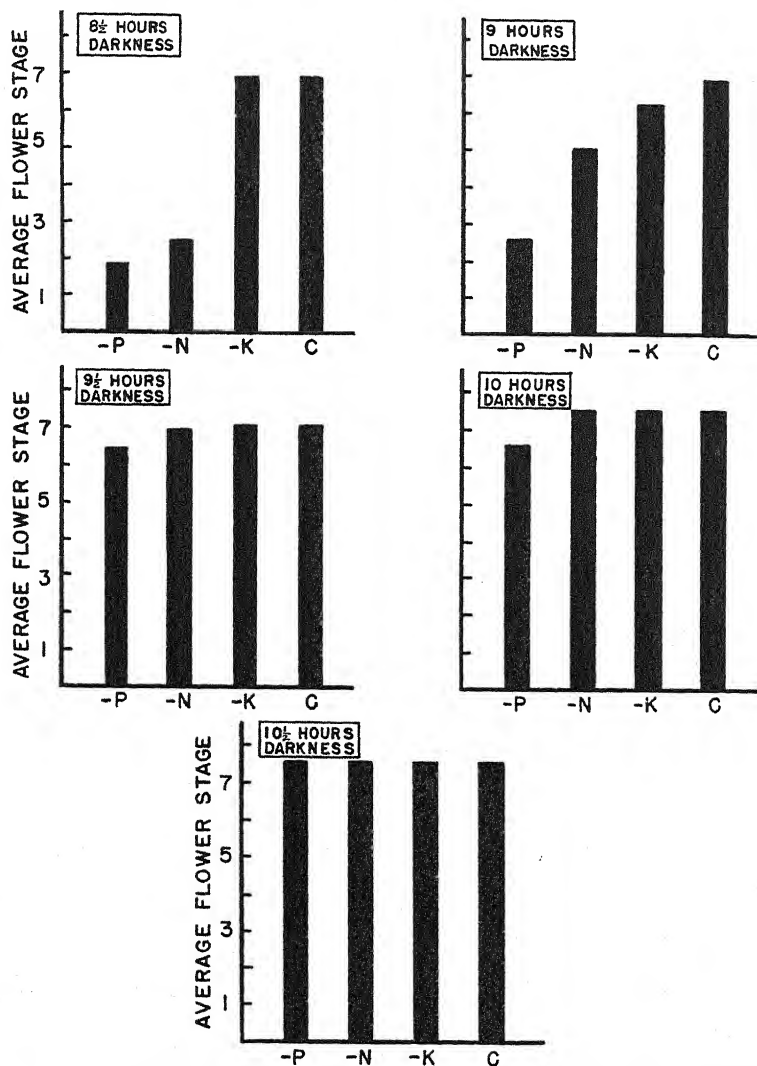


FIG. 1.—Effect of mineral nutrition and number of hours of darkness out of 24 on rate of terminal inflorescence development.

framework chambers where conditions of long photoperiod were maintained. Distilled water was added for 3 successive days. At that time the plants appeared to be in excellent condition.

On June 26, four different types of nutrient solutions were given to each of four different groups of plants in each of the chambers. The four groups (ten plants

each) received complete, $-N$, $-K$, and $-P$ solutions until the end of the experiment. Characteristic deficiency symptoms were beginning to develop when short photoperiod treatment was begun. Starting with July 12, dark periods of the following duration were given in the chambers: 8, $8\frac{1}{2}$, 9, $9\frac{1}{2}$, 10, $10\frac{1}{2}$, and 16 hours. All plants received 8 hours of daylight. The plants were then darkened and supplied in six of the compartments with Mazda filament light of approximately 100 foot-candles at the leaf surface, the length of the light period being automatically controlled by time clocks. In the seventh compartment no supplementary light was used. The plants were harvested 17 days later when floral primordia were macroscopic in the 8-hour photoperiod plus 16-hour dark period group.

The results of this experiment are shown in figure 1. No figures are given for the unit which provided only 8 hours of darkness because all plants were strictly vegetative. Of the units which received $9\frac{1}{2}$ or more hours of darkness, none had any strictly vegetative plants; and there was only one among the 9-hour group.

Plants which received complete nutrients and $-K$ had almost completely formed inflorescences if they were subjected to $8\frac{1}{2}$ or more hours of darkness out of 24. The plants which developed next in rapidity were those low in nitrate; inflorescence primordia of plants deficient in nitrogen, however, developed as rapidly as any others when they had $9\frac{1}{2}$ or more hours of darkness. Those plants showing the slowest rate of development in relation to length of the dark period were those low in phosphorus. Even these plants produced flower primordia as completely developed as under any other condition, provided the length of the dark period was 10 or more hours out of 24. Thus although mineral nutrition apparently does not alter the critical day length of *X. pennsylvanicum*, it does alter to varying degrees—depending upon the number of hours of darkness received—the rate of development of the staminate inflorescences.

CRITICAL DAY LENGTH OF OLD AND YOUNG LEAVES

In a preliminary experiment with 2-month-old plants, none of the leaves were removed. In this instance the leaves selected for experimentation were of two kinds. Half the plants had their first fully expanded leaf, while the other half had one of their very slightly yellowing leaves selected for experimentation. Tops of cardboard cans, painted on the outside with aluminum paint and on the inside with a flat black paint, were slit to the center where a larger opening was made to fit the petiole. The can top was then inserted over the desired leaf of any one plant and tacked firmly to a wooden arm projecting from a right-angle stand. All cut openings were then sealed with adhesive tape or molding clay. The remainder of the can was placed over the leaf blades to provide dark periods varying from 8 to 12 hours out of 24. This single-leaf treatment was continued for 4 days, while the remainder of the plant was on long photoperiod. Subsequently the entire plants received long photoperiods. Dissections made of the terminal bud 5

weeks later indicated merely developmental trends but did not show great uniformity in any one treatment. The indications, however, were that younger leaves could bring about floral induction with shorter dark periods than could older leaves. Also, plants having their young leaves subjected to short photoinductive cycles flowered more vigorously than those having their older leaves so treated.

From this and other experiments (3, 7) it appears possible that the presence of leaves other than those being induced may exert some effect upon the degree of floral response, and that inequalities in area and number of leaves could bring about lack of uniformity in response. Accordingly, an experiment was designed in which all leaves except the one being tested were removed from the plant.

The plants used in this experiment differed from the first in that they were 7 weeks old at the beginning of treatment. Photoperiodic treatment was secured by means of the small chamber and time-clock control system previously described. Two types of controls were employed to determine the effect of different photoperiods on leaf primordia and stems. These consisted of completely defoliated plants in each of the seven chambers and plants maintained on long photoperiod continuously. The different groups of plants received 8 hours of natural daylight plus sufficient Mazda filament illumination to provide photoperiods of 9, $13\frac{1}{2}$, 14, $14\frac{1}{2}$, 15, $15\frac{1}{2}$, and 16 hours out of 24. Treatment was continued for 4 days, the plants then being transferred to long photoperiod, where they remained throughout the experiment.

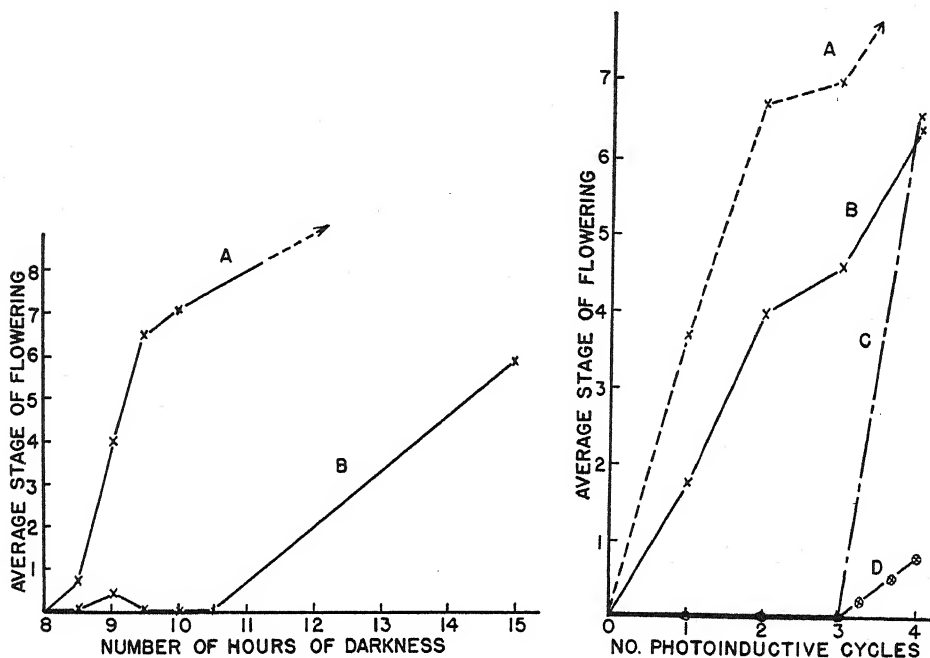
Data given in figure 2 show that the critical dark period for old leaves lies between $10\frac{1}{2}$ and 15 hours, whereas for young leaves it was 9 hours. Thus the young leaves appeared to be much more sensitive to photoinductive treatment than the older ones. Rate of development subsequent to initiation, however, seemed to be directly related to length of the dark period. Figure 2 shows that as the dark-period treatment increased the average stage of inflorescence development at the time of harvest was more advanced.

A possible explanation for the flowering of two plants which received 9 hours of darkness is that the rapidly expanding leaf primordia enlarged sufficiently during experimental treatment to become photoperiodically sensitive. Because the leaves sometimes expanded sufficiently in a day's time to become photoperiodically sensitive, it was necessary to remove the older primordia daily.

EFFECT OF AGE OF LEAVES ON NUMBER OF PHOTOINDUCTIVE CYCLES REQUIRED FOR INDUCTION

In the first experiment, performed to determine the number of photoinductive cycles a single leaf required in order to bring about flowering, not more than one of the leaves was removed from the plant. Inasmuch as the cardboard-can method previously described was being used, it was occasionally necessary to remove one

leaf in order to prepare the expanded leaf for short photoperiod treatment. No strict attempt was made to use either very young or very old leaves; those selected were about midway between the two ages. The twenty plants thus prepared were subjected to four different treatments. Groups of five plants each received 1, 2, 3, or 4 photoinductive cycles consisting of 9 hours of light and 15 hours of darkness. After these treatments the entire plants were left on long photoperiod



FIGS. 2, 3.—Fig. 2 (left), effect of age of leaves of plants which had all except (A) single young leaves and (B) single old leaves removed on rate of development and number of hours of darkness out of 24 required to bring about floral initiation. Fig. 3 (right), rate of floral development of plants which had (A) all except one young leaf removed before the experiment began; (B) single young leaf exposed to short photoperiod, no leaves being removed; (C) all except one old leaf removed before the experiment began; (D) single old leaf exposed to short photoperiod, no leaves being removed.

until one month later. At that time dissection of the terminal buds showed that with one exception the only plants having inflorescence primordia were those which had a single leaf exposed to four photoinductive cycles.

This experiment was followed on June 14 by another in which all the leaves except the first expanded one were removed. Twenty plants were so treated; five which served as controls had all leaves removed. All plants were placed on a truck to receive 9 hours of light followed by 15 hours of darkness. At daily intervals during the treatment five plants with single leaves were removed from the truck and returned to long photoperiod benches. The defoliated controls, to-

gether with the remaining five experimental single-leaved plants, were returned to long photoperiod benches following the fourth photoinductive cycle. During the short photoperiod treatment, expanding leaf primordia were not permitted to enlarge beyond 1 sq. cm. before being removed.

Twenty-six days after the start of the experiment dissections were made. At that time it was found that three of five plants receiving only one photoinductive cycle had flower primordia; four of five plants receiving two photoinductive cycles had flower primordia; all other plants receiving a greater number of photoinductive cycles were flowering.

Because of the apparent differences in behavior of the different aged leaves just noted, an experiment was performed, first to determine the number of days required for induction of old and young leaves when all leaves were present and second to determine the number of days required for induction when only the individual leaves being tested remained on the plants.

For the first part of the experiment eighty uniform vegetative plants were selected and divided into two equal groups. The first fully expanded leaf was selected in one of these groups and old but not yellowing leaves were selected in the second. Triple-thickness black sateen cloth bags were placed over individual leaves at 5:00 P.M. and removed at 8:00 A.M. In order that the bags might not injure the leaves, a thin cane was placed in each pot at such an angle and trimmed to such a length that when the bag was placed over it the open end of the bag reached the base of the petiole. As further precaution against injury, and for stability, the petiole was bound to the cane. After placing the bag over the leaf and end of the cane, its open end was closed around the petiole with a rubber band. Photoinductive cycles were supplied units of ten plants each for 1, 2, 3, or 4 days in both of the age groups. After each unit had received the specified number of photoinductive cycles, the entire plant was maintained on long photoperiod.

Thirty-three days after the experiment was begun dissections were made. The plants which had single young leaves exposed to photoinductive cycles for only one day showed very slight primordial development; those which received a greater number of days of induction showed progressively advanced stages (fig. 3). For the most part, the plants which had had single old leaves exposed to photoinductive cycles were vegetative. None of the plants which had leaves receiving 1, 2, or 3 days of treatment showed any indication of change from the vegetative state, and of those receiving 4 days of treatment only one was definitely producing flower primordia while five others had growing points only slightly changed from the vegetative condition (fig. 3).

In the second experiment, 120 plants were selected for uniformity and placed on a short-photoperiod truck. Of these plants, forty were defoliated except for

the first expanded leaf; forty others were defoliated except for an old leaf; controls consisted of twenty completely defoliated and twenty undefoliated plants. Units of ten plants from each of the single-leaved groups were removed to long photoperiod conditions following each photoinductive cycle. Ten of the undefoliated controls were removed after the second and fourth cycles.

When the plants were harvested 39 days after the experiment began, four of the plants which had a single young leaf exposed to one photoinductive cycle had slightly submacroscopic flowers, four others showed younger stages, and two were vegetative. Those whose leaves received two and three photoinductive cycles all had slightly submacroscopic inflorescences, while those receiving four were macroscopic (fig. 3). In contrast, of those plants which had only one old leaf exposed to photoinductive cycles, none receiving one or two cycles produced flower primordia, only one of ten receiving three cycles showed flower primordia, while all those receiving four cycles had nearly macroscopic inflorescences. All the defoliated controls were vegetative; but of those which had not been defoliated half those receiving one photoinductive cycle had visible inflorescences, the others being almost macroscopic. All those which received two photoinductive cycles had well-developed inflorescences.

Discussion

Considerable work has been done to determine whether flower formation is definitely associated with any one of the mineral elements known to be important in other phases of development. As a result of work with monoecious and dioecious plants, it has been shown that nutrition does affect the degree and direction of floral expression. TIBEAU (19) found that hemp responded to high nitrogen conditions by producing pistillate flowers, while if only a small amount of nitrogen was available his plants were entirely staminate. There is also evidence that highly localized concentrations of nitrogen exert marked effects on development of staminate and pistillate structures of tomato (8), corn, cucumber, and watermelon (12, 20, 18). In addition, NEIDLE (14) has shown that the proportion of staminate and carpellate heads of *X. pennsylvanicum* is dependent upon the carbohydrate-nitrogen relation of the plant and the number of photoinductive cycles received. That other elements may exert some influence upon the development of flowers has been suggested by COMBES (4). There is, however, little evidence to support the theory that floral initiation in photoperiodically sensitive plants results directly from conditions of mineral nutrition. Yet the direction and degree of subsequent development are in most instances markedly affected. This is true also for the tomato, a day-neutral plant, which initiates floral primordia under extremely wide ranges of carbohydrate and nitrogen supply, although subsequent floral and fruit development are sharply limited by the same factors (9).

In the work reported here, it has been shown that even though there was a very low supply of nitrogen, potassium, or phosphorus available, the critical day length of *X. pennsylvanicum* was unaffected. The rate of development of these primordia, especially near the critical photoperiod, depended to a considerable degree upon both phosphorus and nitrogen. The detrimental influence of low supply of these elements on rate of development was overcome, however, by increasing the length of the daily dark period. With increase in the dark period there was an increasing tendency for all primordia to develop at the same rate. Thus if optimum conditions of light and darkness (10 hours of light plus 14 hours of dark) are suddenly imposed, the effect is at least twofold: (a) an initiation of the flower-promoting stimulus and (b) mobilization of both food materials and nutrients at the terminal growing point, even in plants low in nitrogen, phosphorus, or potassium, in sufficient quantities for the rates of development to be approximately the same. NIGHTINGALE (15) has previously shown with tomatoes that, even if they are grown with a nitrogen-free solution, the amount of soluble nitrogen increases if the plants receive long dark periods. This indicates that there may be reutilization of nitrogenous compounds within the plant. Under conditions of increasing dark periods the amount of soluble nitrogen present at the growing tip of a nitrogen-deficient plant would be expected to increase, with the result that the flowers would grow more rapidly. While it may not necessarily be true that phosphorus- and potassium-containing complexes would under similar conditions of increasing darkness be reutilized, the increasing rate of growth of the cells at the apex when the dark period is increased indicates that this is true.

In addition to nutrition, the age and size of the plant may be effective factors in altering response to photoperiodic treatment. BORTHWICK and PARKER reported (1) that flower primordia may be produced at more nodes of older than younger soybeans up to the age of 6 weeks. Moreover the individual leaves possess varying capacity for bringing about the flowering response. Their subsequent investigations have shown that any expanded leaf on a Biloxi soybean plant subjected to photoperiodic stimulation is able to cause the formation of flowers at the growing points. In these experiments (3) they found that—on the basis of the number of flowers produced—the young leaf rapidly increases in ability to stimulate floral initiation until it is fully expanded. Subsequently its capacities to bring about such a response decrease.

While considerable information is available to indicate that growth-regulating substances are produced in relatively young tissues, there is little evidence to show the effects of aging of tissues on their production. Data presented here indicate that old leaf tissue does not bring about nearly as rapid production of the stimulus to floral initiation as do young leaves. There is also some evidence that the

presence of older leaf tissue may have an inhibitive effect upon the rate of development of inflorescence primordia.

It was found that if there are no intervening leaves between the leaf being induced and the stem-tip inflorescence, development proceeds rapidly. On the other hand, in the presence of leaf tissue other than that subjected to photoinductive treatment there is a sharp reduction in the rate of inflorescence development. These findings may be interpreted in at least two ways. It is possible that the flower-promoting substance is destroyed in the stem or leaves, but equally likely that there is a general movement of the stimulus into all leaf tissue, thus permitting little of the stimulus to reach the growing tip. If the stimulus is diverted into the leaf tissue and not destroyed, we should expect the axillary buds to develop into flower primordia. In *X. pennsylvanicum*, however, the terminal inflorescence develops even before the axillary bud in the axil of the induced leaf. Development of axillary buds usually occurs only after the terminal inflorescence is produced. Inflorescences are produced progressively downward from the stem tip (13). Usually the lowest axillary buds do not produce fertile flowers.

In these experiments no evidence was found to support the viewpoint of CHOŁODNY (5) that the flower-promoting stimulus is autocatalytic in action. In every instance it was found that, up to a certain number of photoinductive cycles—depending upon the age of the leaves and the number of leaves present—the rate of development depended upon the number of photoinductive cycles given. HAMNER's work with soybean (6) demonstrated that the number of flowers produced was almost directly proportional to the number of photoinductive cycles given. Thus rate of development and amount of inflorescence development appear to be dependent upon the amount of stimulation given through photoinductive cycles rather than upon continuous autocatalytic formation of the flower-promoting stimulus subsequent to its initiation.

Summary

1. *Xanthium pennsylvanicum* plants low in phosphorus, potassium, and nitrogen had the same critical day length as plants supplied with a nutrient solution containing all these elements.

2. Rate of development of terminal inflorescences of —N, —K, and —P plants was considerably affected at photoperiods near the critical. Lack of phosphorus decreased the rate of development more than lack of either nitrogen or potassium; and lack of nitrogen resulted in slower development than lack of potassium.

3. With each half-hour increase in length of the dark period from $8\frac{1}{2}$ to $10\frac{1}{2}$ hours there was an increasing tendency for the plants low in phosphorus, nitrogen, or potassium to develop at the same rate as the plants grown with a non-deficient solution. Thus it appears that by increasing the length of the dark period

the detrimental effect of mineral deficiency on rate of development of floral primordia may be overcome.

4. Age of leaf tissue definitely affects the critical day length. The first fully expanded leaf had a critical dark period of 9 hours, whereas in the older leaves it was between $10\frac{1}{2}$ and 15 hours. Rate of development subsequent to initiation was dependent on the length of the dark period.

5. Single young leaves may bring about induction to flower if given only one photoinductive cycle, but the flowering response is more pronounced if more cycles are given. Four photoinductive cycles brought about as rapid rate in inflorescence development in the plants defoliated except for one leaf as two cycles in the undefoliated plants. Old leaves, on the other hand, required four photoinductive cycles to bring about a change from the vegetative state.

6. The data substantiate the theory that the presence of leaf tissue other than that being photoperiodically induced decreases the effectiveness of the flower-promoting stimulus in bringing about floral initiation and the subsequent rate of development.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Effectiveness of photoperiodic treatments of plants of different age. *BOT. GAZ.* 100:245-249. 1938.
2. ———, Effect of variations in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. *BOT. GAZ.* 101:145-167. 1939.
3. ———, Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. *BOT. GAZ.* 101:806-817. 1940.
4. COMBES, R., Action du milieu sur la nutrition azotée de la fleur. *Compt. Rend. Acad. Sci. (Paris)* 206:1980-1982. 1938.
5. CHOLODNY, N. G., The internal factors of flowering. *Herbage Reviews* 7:223-247. 1939.
6. HAMNER, K. C., Interrelation of light and darkness in photoperiodic induction. *BOT. GAZ.* 101:658-687. 1940.
7. HAMNER, K. C., and BONNER, J., Photoperiodism in relation to hormones as factors in floral initiation and development. *BOT. GAZ.* 100:388-431. 1938.
8. HOWLETT, F. S., The effect of carbohydrate and of nitrogen deficiency upon microsporogenesis and the development of the male gametophyte in the tomato, *Lycopersicum esculentum* Mill. *Ann. Bot.* 50:767-804. 1936.
9. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. *Oregon Agr. Exp. Sta. Bull.* 149. 1918.
10. LONG, E. M., Photoperiodic induction as influenced by environmental factors. *BOT. GAZ.* 101:168-188. 1939.
11. MANN, L. K., Effect of some environmental factors on floral initiation in *Xanthium*. *BOT. GAZ.* 102:339-356. 1941.
12. MININA, E., and GUSEVA, V., Vliianie mineral' nogo pitamiia na priznaki pola kukuruzy.

- (The influence of mineral nutrition upon the sex characteristics of corn.) Chem. Soc. Agr. 3:47-60. 1937.
13. NAYLOR, FRANCES L., Effect of length of induction period on floral development of *Xanthium pennsylvanicum*. BOT. GAZ. 103:146-154. 1941.
 14. NEIDLE, EDITH K., Nitrogen nutrition in relation to photoperiodism in *Xanthium pennsylvanicum*. BOT. GAZ. 100:607-618. 1938.
 15. NIGHTINGALE, G. T., The chemical composition of plants in relation to photoperiodic changes. Wisconsin Agr. Sta. Bull. 74. 1927.
 16. SNYDER, W. E., Effect of light and temperature on floral initiation in cocklebur and Biloxi soybean. BOT. GAZ. 102:302-322. 1940.
 17. STEINBERG, R. A., and GARNER, W. W., Response of certain plants to length of day and temperature under controlled conditions. Jour. Agr. Res. 52:943-960. 1936.
 18. TAPLEY, W. T., The fruiting habit of squash. Proc. Amer. Soc. Hort. Sci. 20:312-319. 1923.
 19. TIBEAU, M. E., Time factor in utilization of mineral nutrients by hemp. Plant Physiol. 11: 731-747. 1936.
 20. TIEDJENS, V. A., Sex ratios in cucumber flowers as affected by different conditions of soil and light. Jour. Agr. Res. 36:721-746. 1928.

MORPHOLOGICAL STUDY OF AGAVE LECHUGUILLA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 534

ALVIN R. GROVE

(WITH THIRTY-EIGHT FIGURES)

Introduction

STENAR (6) has compiled much of the literature of the Amaryllidaceae published previous to 1925. His discussion of the Agavoideae is brief, and much of his information is based on ERNST's work on *Fourcroya cubensis*. SCHAFFNER (3) and OSTERHOUT (2) have reported some work on pollen development, with special emphasis on the cytological investigation of microspore mother cell divisions and chromosome counts. SCHNARF (5) discusses in some detail the reproductive habits of some genera of the Amaryllidaceae, but statements concerning the genus *Agave* are meager and based again in large part on ERNST's work on *Fourcroya*. SCHLIMBACH (4), in discussing the Amaryllidaceae, makes general reference to the family but does not present details concerning *Agave*. GOEBEL (1) makes several short references to this genus, speaking in particular of its hollow style and the development of a multilayered endothecium in *A. americana*.

The research reported here includes studies of the origin and development of the floral organs and their relation to the axis and bracts which become involved in such development; the vascular anatomy of the flower; the development of the ovule; megasporogenesis and megagametogenesis; and the development of the anther. No effort has been made to count the chromosomes or to make a cytological investigation of the nuclear divisions. The present investigation started with actual flower formation, but much growth of the flowering axis has already preceded this stage. Elongation of the axis from the center of a basal rosette of foliar leaves is the first indication of flowering. This stalk elongates rapidly and may reach a height of many feet before recognizable flowers appear. The material studied was collected from the tip of this axis after its elongation had taken place.

Material and methods

Material of *Agave lechuguilla* Torr. was collected in the field near El Paso, Texas, May 7, 1939, and June 1, 1940, and also at Alpine, Texas, May 5, 1939. Flowers and developing fruits were dissected from healthy plants and were killed and fixed in Sax's modification of Navashin's solution. The material was separated into approximately twenty age groups, from tips to oldest fruits, and later sectioning was done on random samples from these groups. Treatment was according

to the paraffin method, and sectioning was at 3-15 μ . Flemming's triple stain and light green and safranin were employed. At the time that material was fixed for sectioning, other material was killed in formalin-acetic-alcohol and used for gross study.

Observations

ORIGIN AND DEVELOPMENT OF FLOWER

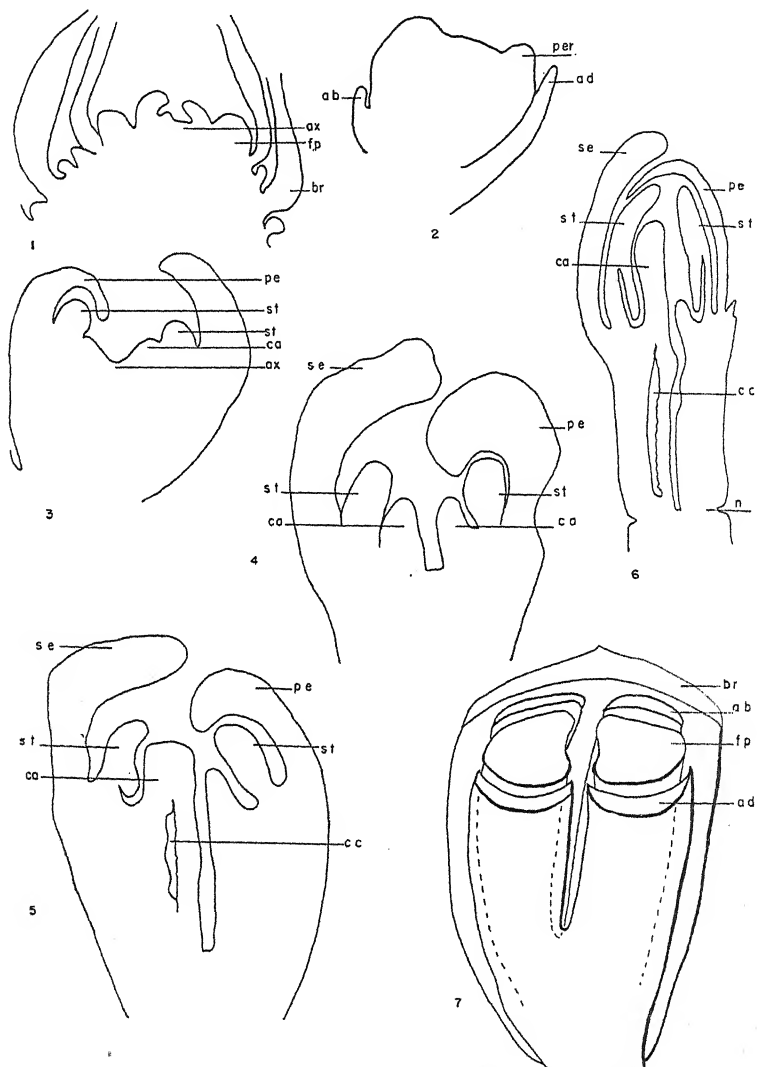
The first evidence of floral development is the appearance of a single branch primordium in the axil of each bract, referred to here as the outer bract, diverging from the main axis of the plant (figs. 1, 7). Before appreciable elongation of this branch primordium has taken place, two bracts, referred to here as the adaxial flower bracts, arise near its base and toward the axis. As development of these bracts takes place, their bases elongate conjointly and at first only their tips are separate. Later, after the flowers themselves are well developed, the conjoint base splits so that apparently two completely separate bracts are present, one on the adaxial side of each flower branch. Before any elongation of the first branch primordium has taken place a second one arises in the axil of the bract on the first branch primordium. In a cross section of the young flowering axis these two primordia lie inside the outer bract and outside the conjoint base of the two developing adaxial bracts (fig. 7). The origin of the first and second branch primordia and the two adaxial bracts can be determined from cross sections in which the bases of all four structures are common. Later, as elongation takes place, each can be seen as a separate structure near its tip. The first and second branch primordia are completely covered with an epidermis, as are both adaxial bracts. In addition to the structures already mentioned, each flower primordium bears a bract on its outer face, the abaxial bract. These bracts develop later than the adaxial bracts, and flowers may or may not develop in their axils. This development usually leads to the formation of two flowers, one differentiated at the tip of the first branch primordium and one at the tip of the second. Not always, however, does development stop with two flowers, and cases can be found where three or even four ultimately develop. Such a branch system of flower origin would seem the normal behavior for the plant and accounts for the origin and variation in number of flowers present.

PERIANTH AND SEPALS

The perianth arises first as a ring on the periphery of the flower primordia. Not long after, the three sepals arise from this as separate primordia. At this time there is much lateral growth of the end of the branch, which causes it to widen and flatten (fig. 2). Subsequent elongation of the sepal primordia (figs. 3-6) leads to three elongated and flattened sepals which are widened at their base and which in the mature flower overlap the petals to some extent.

PETALS

The petal primordia, three in number, arise next in order and lie just inside the ring of sepal primordia and alternate with them (fig. 3). They eventually elongate



FIGS. 1-7.—Figs. 1-6, successive development of floral parts. Fig. 7, reconstruction showing relation of bracts to flower primordia; axis removed. (*ax*, axis; *fp*, flower primordia; *br*, outer bract; *ab*, abaxial bract; *se*, sepal; *pe*, petal; *ca*, carpel; *st*, stamen; *cc*, carpellary cavity; *n*, node.)

and are sepaloid in appearance (figs. 4-6), contain chlorophyll, and are not showy. In shape they are similar to the sepals and approximate them in size. In the mature flower their edges are overlapped by alternating edges of the sepals.

STAMENS

Two rings of three stamens each are next in origin. The outer ring of three lies opposite the three sepals and the inner ring of three within and opposite the three petals. The primordia arise as more or less dome-shaped protuberances, and all six stamens develop simultaneously in two cycles (fig. 3). At the time the stamens originate the sepals are already well developed and the petals are rapidly elongating. In later stages of development the anther arises as a terminal expansion of the stamen. Its growth is parallel to the filament, and in the mature condition the filament is attached at about the center of the elongated anther, being versatile. When the flower opens at time of pollination the anther flips up, its median attachment to the filament operating as a hinge (fig. 6).

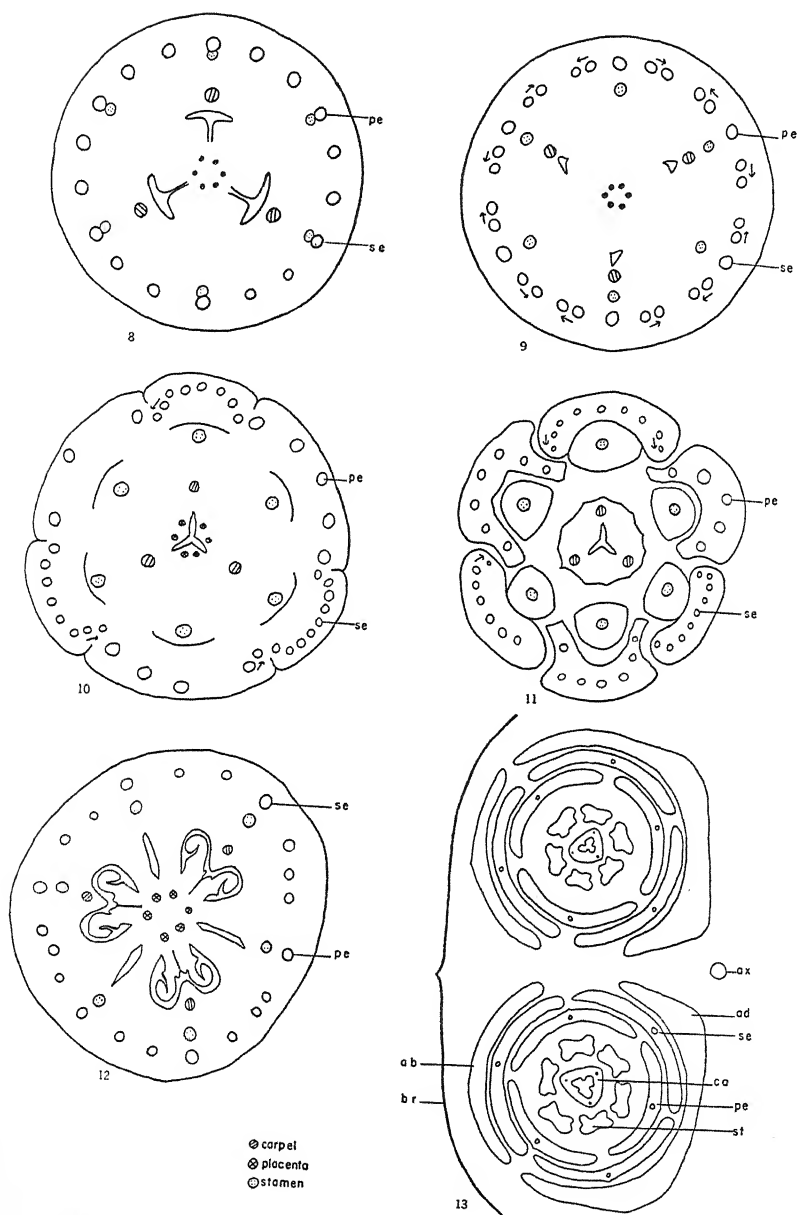
CARPELS

The three carpel primordia arise last and opposite the sepals, and, like the stamens, they are at first more or less dome shaped (fig. 3). At this stage in ontogeny the growth of the end of the axis is inhibited, but all the conjoint flower parts continue to elongate and enlarge above it, so that in the young flower a canal is left in the center of the developing ovary. However, the conjoint development of the three carpels forms a common central tissue which completely fills this central canal in the mature flower (fig. 12) and leads to the formation of a compound ovary with three separate and completely inclosed carpellary cavities. Zonal elongation of the conjoint bases of the three carpels completes the formation of the compound pistil. The tips of the three carpels continue above the ovary as a compound style, which is three lobed and hollow. As zonal elongation of the three carpels takes place, zonal elongation of the undiverged bases of the sepals, petals, and stamens also occurs. This conjoint tissue completely surrounds and is indistinguishable from the carpels (fig. 12).

The general development just described is one of acropetal succession, starting with sepals and progressing inward toward the center of the flower through petal, stamen, and carpel.

VASCULAR ANATOMY OF FLOWER

A node at the bottom of the ovary makes it difficult to trace the vascular supply of the flower down through the flower branch (fig. 6). In a very young flower, before differentiation of the majority of the procambial strands, it is possible to trace a certain amount of vascular tissue through the node, but since all traces are not differentiated, the lack of obvious nodal ramifications complicates the interpretation rather than simplifies it. For this reason no attempt has been made in the drawings to trace the vascular supply through the node; only the bundles above it after vascular tissue differentiation has taken place have been recorded (figs. 8-13). In no instance have drawings been included at the tips of sepals, petals, etc.



FIGS. 8-13.—Figs. 8-11, successive cross sections at representative levels. Fig. 12, cross section of older ovary showing relation of ovules. Fig. 13, diagrammatic cross section of two flowers, showing orientation of floral members. (*se*, sepal; *pe*, petal; *ax*, axis; *ad*, adaxial bract; *ab*, abaxial bract; *br*, outer bract; *ca*, carpel; *st*, stamen.)

At the base of the ovary there is visible a median bundle for each sepal and each petal. In addition there are twelve lateral bundles, one on either side of each median sepal and petal bundle. On the inner side of each median sepal and petal bundle is a stamen bundle. In most cases at this level this bundle is so closely appressed that it is difficult to distinguish it. Three dorsal carpel bundles are present at this level and remain unchanged throughout the upper extent of the flower, passing into the style at the top of the ovary. Six placental bundles are present, but at this stage show little differentiation (fig. 8).

Examination of cross sections of the flower at higher levels shows that the median bundle of each sepal passes into the sepal without dividing. Each lateral bundle is divided to give rise to one toward the edge of the sepal. The bundles formed by this first division divide, and the derived bundles here divide once. This leads to the usual condition of nine bundles present in the sepal after its divergence at the top of the hypanthium (fig. 11). The petal bundles show the same kind of development, except that two divergences usually occur and seven bundles rather than nine is the usual number present in the petal (fig. 11). The stamen bundles, as already mentioned, lie inside of and are tightly appressed to each median sepal and petal bundle (fig. 8). At the top of the hypanthium the stamen bundles pass directly up into the stamens (fig. 11).

The vascular plan above the node is a simple one, and indicates that the hypanthium consists of the undiverged bases of sepals, petals, and stamens, as shown by their vascular anatomy.

OVULE DEVELOPMENT

The nucelli arise from the lateral edges of each of the three carpels. As growth continues the nucellus elongates, and with growth more rapid on the outer face the nucellus gradually turns through an arc of nearly 180° to become anatropous. Two integuments are developed from the base of the nucellus and ultimately overgrow its end to form the micropyle. The outer integument is fully developed only on the outer face of the ovule and is present only as a ring on the funicular side.

Before any appreciable curvature of the nucellus, and previous to the origin of the inner integument, the archesporial cell is differentiated. The megaspore stages are numerous when the nucellus has passed through about a 90° arc, and one integument is developed—with the second starting in some instances. Megagametophyte stages occur when the ovule has assumed its complete anatropous position and both inner and outer integuments are well developed.

MEGASPOROGENESIS

A single hypodermal cell enlarges from the sporophytic tissue of the nucellus. It can readily be identified by its position, immediately beneath the epidermis; its increased size and denser cytoplasm; and its enlarged nucleus. It is differentiated

before the appearance of the inner integument and before any appreciable growth of the nucellus has taken place (fig. 14). The first and only division of the archesporial cell is periclinal, resulting in the primary sporogenous cell on the inside and the primary parietal cell on the outside (fig. 15). The primary parietal cell is divided in turn by an anticlinal division (fig. 16) and eventually gives rise to a single parietal layer (figs. 17-20). The parietal tissue persists until the megagametophyte starts to enlarge, at which time it is crushed.

The primary sporogenous cell enlarges directly into the megaspore mother cell (fig. 17). Two divisions of this cell and its derivatives follow in rapid succession, and a linear tetrad of four megaspores is formed (figs. 18, 19). Not until both divisions are completed is there evidence of wall formation, but following the second division walls come in to form four cells (fig. 20). Although chromosome counts were not made, it is assumed that these two divisions involve reduction of the chromosomes from the diploid to the haploid number. Of the linear tetrad so produced the megaspore toward the chalazal end of the ovule survives and the remaining three disintegrate (fig. 20). This results in an enlarging uninucleate cell which becomes directly the young megagametophyte (fig. 23).

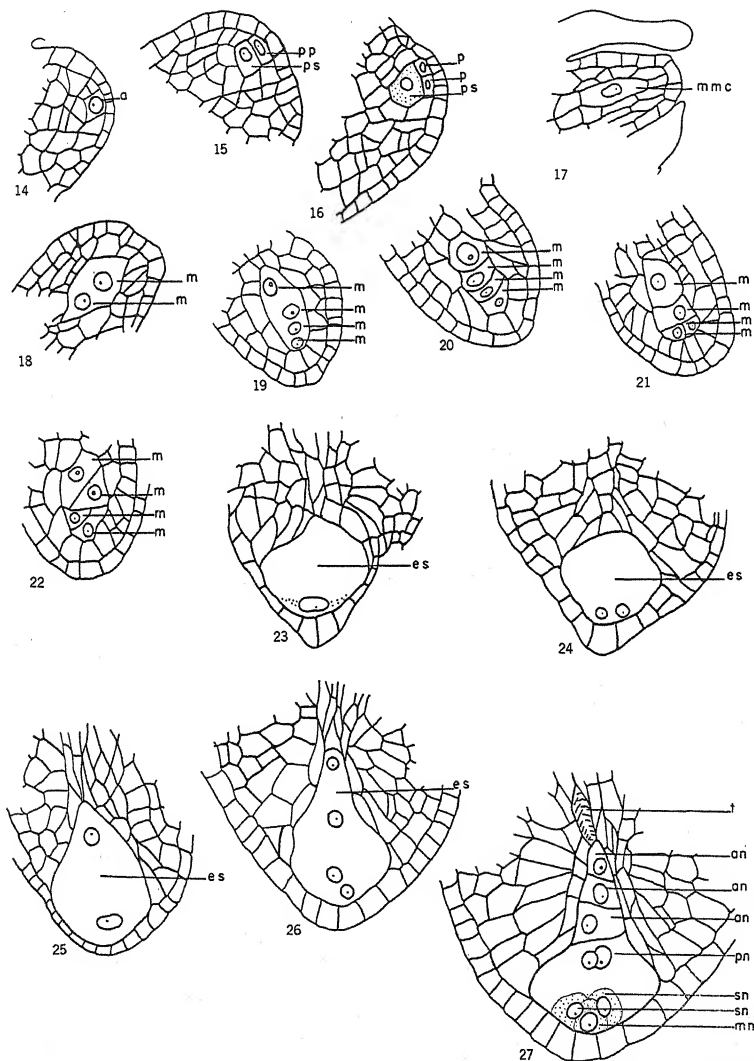
VARIATION IN MEGASPORE ARRANGEMENT

In addition to the usual linear tetrad arrangement of the four megaspores, two variations were noted rather commonly. One variation is essentially the same as the linear tetrad, except that the walls between the megaspores come in at angles to one another instead of parallel (fig. 22). A second variation shows the wall between the two micropylar nuclei at right angles to the wall between the other two toward the chalazal end (fig. 21). This is similar to a condition already recorded for *Haemanthus katharinae* and *Bomarea caldasii* (6).

MEGAGAMETOPHYTE DEVELOPMENT

The single nucleus of the enlarging young megagametophyte is located near the micropylar end (fig. 23). It divides to give rise to two nuclei (fig. 24) and one nucleus, then migrates toward the chalazal end (fig. 25). The cytoplasm between these two nuclei, one the primary chalazal and the other the primary micropylar, becomes highly vacuolated as enlargement occurs. Each of these two primary nuclei divides to give rise to a four-nucleate megagametophyte (fig. 26). Division of the primary micropylar nucleus precedes that of the primary chalazal nucleus. Each of the four nuclei now present undergoes one more division, giving rise to eight. Of the four at the micropylar end of the megagametophyte, one develops as the egg, two as synergids, and one as a polar nucleus. Three of the four nuclei at the chalazal end become nuclei antipodal cells arranged in linear manner and one a polar nucleus. The two polar nuclei migrate toward the center of the megagametophyte.

tophyte and together make the primary endosperm nucleus, fusion not taking place until fertilization or soon thereafter. The egg and each synergid have a defi-



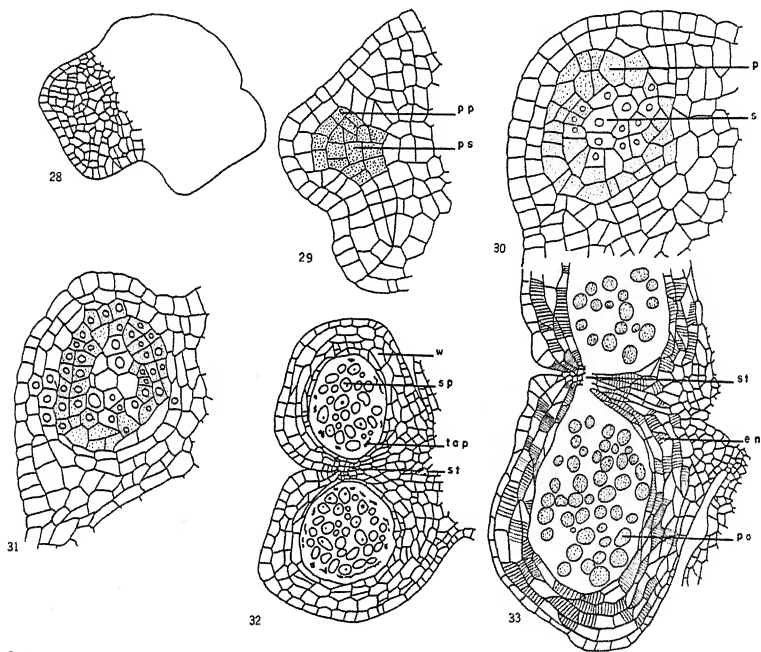
FIGS. 14-27.—Megasporogenesis and megagametogenesis. (*a*, archesporial cell; *pp*, primary parietal; *ps*, primary sporogenous; *p*, parietal; *mmc*, megaspore mother cell; *m*, megaspore; *es*, embryo sac; *t*, tracheid; *an*, antipodal nucleus; *pn*, polar nuclei; *sn*, synergid; *mn*, megagamete nucleus.)

nite wall and a fairly large amount of cytoplasm. The cell containing the two primary polar nuclei is much larger than any of the others (fig. 27). The cytoplasm of the two synergid cells cups slightly around the egg cell, which takes a central position between the two synergid cells.

Instead of the vascular tissue ending at the base of the chalaza, tracheids are often differentiated in the tissue between the chalaza and the megagametophyte itself. Apparently there are only tracheids and there is no indication that phloem elements are present also. This condition is rare in plants (fig. 27).

MICROSPORES AND MICROGAMETOPHYTE

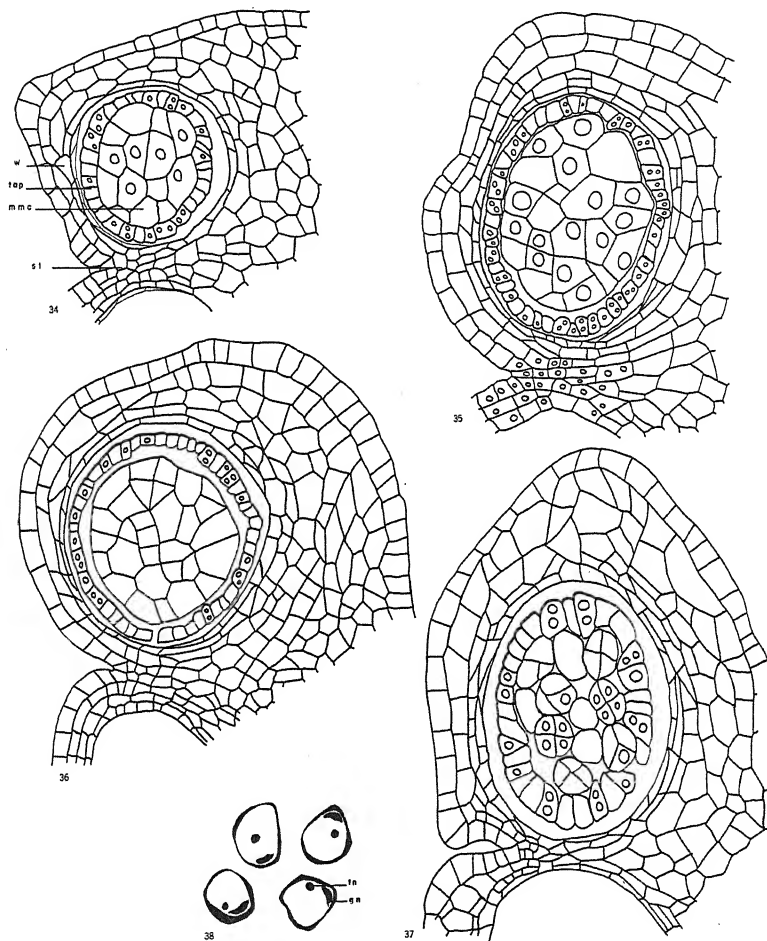
The anther, when mature, is terminal and versatile. As its development proceeds, four distinct lobes are formed. At first, all the cells of these lobes, exclusive of the epidermis, are similar in size, in density of cytoplasm, and in general appearance (fig. 28).



FIGS. 28-33.—Anther development. Figs. 28-31, early development of parietal and sporogenous tissue. Fig. 32, microspore separation. Fig. 33, mature anther. (*ps*, primary sporogenous; *pp*, primary parietal; *w*, wall; *st*, stomium; *en*, endothecium; *sp*, spores; *po*, pollen; *tap*, tapetum; *s*, sporogenous; *p*, parietal.)

The archesporium differentiates as four groups of cells from hypodermal tissue, one in each lobe of the anther. The archesporium is not easily distinguished at first, but by the time of periclinal division of these cells in the formation of primary parietal and primary sporogenous tissue it is easily distinguished (fig. 29). The primary parietal tissue thus formed undergoes several other periclinal and random anticlinal divisions in the formation of wall tissue which surrounds the more or less centrally located sporogenous mass (figs. 29-31). The innermost layer of parietal cells differentiates as tapetum. The cells of the tapetal layer are small at first but

gradually enlarge, and by the time microspore mother cells are recognized many of the tapetal cells are binucleate. Although the tapetum is fully formed by the time microspore mother cells are differentiated, further enlargement of the tapetal



FIGS. 34-38.—Anther development. Fig. 34, microspore mother cells. Fig. 35, at time of first reduction division. Fig. 36, at time of second reduction division. Fig. 37, separation of spores. Fig. 38, mature pollen. (*mmc*, microspore mother cell; *tap*, tapetum; *w*, wall; *st*, stomium; *tn*, tube nucleus; *gn*, generative nucleus.)

cells does take place and its greatest development is simultaneous with the formation of microspores. From this period on the tapetum disappears and the microspores enlarge and develop. In some instances at least, disintegration of the tapetum supplies a nutritive mass which surrounds many of the newly separated spores. The tapetum and its remnants continue for a long time during spore de-

velopment, but when mature pollen grains are found it has entirely disappeared (figs. 34-37).

The remaining wall cells derived from the parietal tissue stay more or less intact, although there may be some disintegration of the inner layer as the tapetum enlarges. When the anther matures the remaining wall tissue is characterized by the formation of a well-defined endothecium, which involves all but the epidermal layer of wall tissue (fig. 33).

The primary sporogenous tissue is formed by division of the archesporium. These primary sporogenous cells may undergo one or more divisions and the resulting cells enlarge and function as microspore mother cells (figs. 30, 34). The microspore mother cell undergoes two successive divisions, the reduction divisions, and wall formation follows each immediately, forming four microspores (figs. 34-36). Following the second division the microspores separate, usually in groups of four cells, following the pattern of the original microspore mother cells; but these groups soon separate, the individual spores becoming independent. They are at first thin walled and uninucleate and may in part be surrounded with a cytoplasmic mass from the disintegrating tapetal cells (figs. 32, 37). As the microspores mature their wall continues to thicken, and division of the nucleus results in the formation of a two-nucleate microgametophyte. The tube cell is large and occupies the greater part of the pollen grain. In cross sections of mature pollen grains one thin place is always recognized in the wall surface surrounding the tube cell.

The generative nucleus is vermiform and its cell is considerably smaller and less regular in outline than the tube cell (fig. 38). Stomium structure is traced in figures 28-37. As the anther matures, with pollen grains and endothecia differentiation, the stomium breaks with little decomposition and the locular spaces of the two adjoining pollen groups become one (fig. 33).

Summary

1. Origin of the floral organs of *Agave lechuguilla* is acropetal, sepals appearing first and carpels last.
2. The flower is epigynous. Its vascular anatomy is simple, being a direct divergence of vascular bundles into the separate organs. All vascular bundles ending in either sepal or petal structures are derived from bundles differentiated early in connection with these floral parts.
3. The ovule is anatropous and has two integuments. Placentation is axial.
4. Megasporogenesis starts with differentiation of a single hypodermal archesporial cell and ends with formation of a tetrad of megaspores arranged usually in linear fashion. Two variations in megaspore arrangement were noted.

5. Periclinal division of the archesporial cell gives rise to a primary parietal cell and to a single primary sporogenous cell which enlarges and becomes the megaspore mother cell. Development of the megagametophyte follows the so-called normal type.

6. A single parietal layer is present in the ovule, disintegrating when the megagametophyte enlarges.

7. The vascular tissue of the ovule does not stop at the base of the chalaza but tracheids penetrate the nucellus to the megagametophyte.

8. Anther development follows the common method. The archesporium consists of a group of cells. Periclinal divisions give rise to the primary parietal and primary sporogenous cells. Continued periclinal and anticlinal divisions of the primary parietal cells form the wall tissue. The sporogenous cells enlarge and function as microspore mother cells. There are two successive divisions of the microspore mother cells, resulting in four microspores.

9. The tapetum is derived from the innermost layer of the parietal tissue.

10. The mature anther shows well-defined endothecia three or four cells in thickness and disintegration of the stomium.

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LITERATURE CITED

1. GOEBEL, KARL, Outlines of classification and special morphology of plants. Engl. Transl. by H. E. F. GARNSEY. 1887.
2. OSTERHOUT, W. J. V., Cell studies. I. Spindle formation in *Agave*. Proc. Calif. Acad. Sci. Bot. Series 3. 2:255-284. 1904.
3. SCHAFFNER, J. H., The reduction division in the microsporocytes of *Agave virginica*. BOT. GAZ. 47:198-214. 1909.
4. SCHLIMBACH, H., Beitrage zur Kenntnis der Samenanlagen und Samen der Amaryllidaceen mit Berucksichtigung des Wassergehaltnes der Samen. Flora 117:41-54. 1924.
5. SCHNARF, K., Vergleichende Embryologie der Angiospermen. 1931.
6. STENAR, A., Embryologische Studien I, II. II. Die Embryologie der Amaryllideen. 1925.

EFFECT OF VARIOUS GROWTH-REGULATING SUBSTANCES UPON SEVERAL SPECIES OF PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 535

DAPHNE B. SWARTZ

Introduction

Many recent investigations have been concerned with attempts to determine the functions of certain of the phytohormones. Notable among these substances are those—such as vitamin B₁ and nicotinic acid—which have been shown, in experiments with isolated embryos and excised roots, to be essential to root development (1, 8). These experiments indicate also an interdependence of these two substances. For example, BONNER (1, 2) found that while in certain instances nicotinic acid alone was not effective, when applied in addition to vitamin B₁ it intensified the response of the plant to the latter.

But whether these substances, under certain conditions, may become limiting factors to the extent that their external application will affect the total plant growth, is still a question. BONNER and GREEN (3) have reported increased dry weight of both roots and shoots, accompanying increased general vigor, when vitamin B₁ was supplied with nutrient solution to plants in sand cultures. In marked contrast to these results are those reported in the recent work of HAMNER (6). Under variously controlled conditions of photoperiod and nitrogen supply, plants supplied with vitamin B₁ showed no increased dry weight. GORHAM (5) also reports no increased frond multiplication in *Lemna* when vitamin B₁ was added to the solution in which it was growing.

Other substances, such as naphthaleneacetic acid and related synthetic compounds, though not yet known to be formed in plants, are known to increase the rate of root initiation when applied to stems, leaves, and plant parts other than roots, in general (9). These same substances, which initiate root formation, may fail to promote growth and even in high dilutions inhibit root growth (4). Somewhat at variance with this is the report of GORHAM (5) that concentrations of 0.25 and 0.10 mg. of naphthaleneacetic acid per 100 ml. of nutrient produced marked increase in the rate of frond multiplication in *Lemna*. It has also been well established that pronounced histological changes accompany root formation and other external changes induced by naphthaleneacetic acid (7). But the effect which these root-forming substances may have upon total plant growth (as indicated by dry weight), and the external conditions under which these effects may occur, are as yet largely undetermined.

The present investigation, except for a part of the preliminary experiment, attempted only to determine the effects resulting from the addition of various growth substances to plants supplied with a complete nutrient solution to the sand medium in which they were grown. Great numbers of plants were used to make statistical analysis of the results possible.

Investigation

CHRYSANTHEMUM

This experiment was conducted, in part, to determine whether the addition of a growth substance may in any measure compensate for the lack of an essential element in the nutrient solution.

On April 26, chrysanthemum cuttings which had been rooted and were of approximately uniform size were transplanted into 4-inch glazed pots with side drain-

TABLE 1
DRY WEIGHT* IN GRAMS OF CHRYSANTHEMUM PLANTS

	I COMPLETE NUTRIENT		II MINUS Mg NUTRIENT		III MINUS K NUTRIENT		IV MINUS P NUTRIENT	
	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS	TOPS	ROOTS
Control.	4.72	2.11	0.81	0.17	0.4	0.16	0.17	0.21
Vitamin B ₁ added.	4.59	2.03	0.95	0.24	0.55	0.28	0.15	0.21
Naphthaleneacetic acid added.	4.21	1.94	0.72	0.20	0.8	0.39	0.16	0.31

* Weights represent mean of fifteen plants grown under each treatment.

age. The pots had been previously filled with quartz sand and flushed with distilled water. They were supported on lath racks to prevent any contamination of the nutrient supply through drainage. Fifteen plants were provided for each of the four treatments (table 1), and the pots were placed at random on the greenhouse bench in rows of five each. During the 10 weeks which followed, the plants were supplied with nutrient solution, with added growth substances on alternate days, and with distilled water on intervening days. Once a week the pots were thoroughly flushed with distilled water. The complete nutrient solution used in this, as well as in the more extensive experiments which followed, was made up from the following 0.5M stock solutions. Of these there were added to 72 liters of distilled water: 864 ml. of $\text{Ca}(\text{NO}_3)_2$, 648 ml. of MgSO_4 , and 648 ml. of KH_2PO_4 . In the "minus" solutions, the following substitutions were made: NaH_2PO_4 for KH_2PO_4 in the minus K solution, KCl for KH_2PO_4 in the minus P solution, and Na_2SO_4 for MgSO_4 in the minus Mg solution. Minor elements were supplied in

the following parts per million of nutrient: boron 0.5, manganese 0.5, zinc 0.05, and copper 0.02. A 0.5 per cent iron tartrate solution was added once weekly at the rate of 1 ml. per liter of nutrient. The growth substances, vitamin B₁ (Merck) and naphthaleneacetic acid, were mixed with the nutrient solution immediately before it was supplied to the plant, each being prepared in aqueous stock solution and used in the dilution of 0.1 mg. per liter of nutrient.

The chrysanthemum plants were harvested on July 10. The roots were separated from the tops at the soil line, and the roots were washed free of adhering sand by dipping them in salt water and then rinsing in distilled water. After being blotted to remove the excess water, they were placed in open paper containers and dried to constant weight in a draft oven. Weights were taken immediately after the plants cooled.

COSMOS AND MARIGOLD

Plants used in this more extensive investigation were chosen because they grow well under the variable conditions of temperature and light which prevail in the Chicago area during the summer months. Both the Harmony marigold and the Burpee Golden cosmos have a comparatively short vegetative period before flowering. And this variety of cosmos has been used in previous experiments of similar nature.

Four-inch glazed pots like those of the previous experiment were used. Quartz sand which had been washed and sifted to free it of organic matter was used. On June 6, selected seeds secured from the Burpee Company of Philadelphia were planted, four or five in each pot at uniform depth. One hundred thirty pots were planted for each of the four treatments of both varieties of plants used, making 1040 pots in all. They were placed at random in rows of ten each on two greenhouse benches.

Germination was almost 100 per cent. Soon after emergence, all but two seedlings were removed from each pot. Within 2 weeks the second seedling was removed, leaving only one in each pot. At this stage, plants of both varieties were very uniform in height and vigor. Complete nutrient solution was supplied twice during the first week after emergence. Beginning on the tenth day, and continuing throughout the course of the experiment, growth substances were added to the nutrient solution and supplied to the plants twice a week. Plants of one group served as controls and received only the complete nutrient solution; plants of another group received naphthaleneacetic acid added to the nutrient solution; a third group received nutrient plus nicotinic acid; and a fourth group received both nicotinic acid and vitamin B₁ in addition to the nutrient solution. All the growth substances were used in the dilution of 0.01 mg. to 1 liter of nutrient. Both marigolds and cosmos were harvested after 8 weeks. The methods used in harvesting and drying were the same as those previously described.

Results and discussion

All chrysanthemum plants of group 1 (table 1), whether or not they were supplied with growth substances in addition to the complete nutrient solution, showed similar vigor. They were very uniform in height and averaged approximately the same number of leaves. The plants which received minus Mg solution showed no detectable variation. All plants, regardless of growth substances, were definitely stunted and showed typical magnesium deficiency. Average dry weight of tops of plants receiving B₁ was slightly greater than that of the controls.

The only group which evidenced any marked response to the addition of growth substances was the one receiving minus K nutrient solution. All plants showed typical potassium deficiency, with browning and final abscission of the leaves. Both the plants receiving B₁ and those receiving naphthaleneacetic acid were more vigorous than the controls, although there was only a slight increase in height. The difference was most apparent in plants which received naphthaleneacetic acid. They showed a significant increase in dry weight. They tended to retain ten or twelve leaves in contrast to an average of nine in the controls, and the leaves were somewhat larger. There was also a tendency for the plants receiving naphthaleneacetic acid to produce one or more stolons. None was formed on the control plants.

The numbers of plants involved in the experiment were too small to justify definite conclusions, but their response gives evidence of the possibility that naphthaleneacetic acid may in some degree compensate for lack of potassium. As previously stated, the experiment with cosmos and marigold constituted the major portion of this investigation. Tables 2 and 3 show little response of the plants to the growth substances. The most striking effect was the marked individual variation in height among the cosmos plants. Each group, regardless of treatment, showed a variation in height from a minimum near 20 cm. to a maximum of over 90 cm. All plants seemed vigorous and healthy, regardless of height. This marked variability illustrates how much an inconstant characteristic might be erroneously attributed to some external factor, such as a growth substance, if plants were observed in small numbers.

Table 2 shows an accompanying variation in dry weight occurring within each group of cosmos plants. A statistical analysis to determine the significance of the variances indicates that the mean differences in weight occurring between each group and the control (table 4) lie well below the mean calculated as significant on the basis of 5 per cent probability. Comparison of the cosmos leaves showed a similar range of size in all groups. No variation in floral initiation among the groups was noted. At the time of harvest, 79 out of 130 control plants were in bud or blossom as compared with 74 in the naphthaleneacetic-acid group, 73 of those receiving nicotinic acid, and 77 receiving both nicotinic acid and B₁.

Growth of the marigolds was much more uniform. Approximately the same number of plants from each group showed floral initiation within 5 weeks. After the terminal flower had formed, axillary shoots developed with noticeable rapidity in all plants. Dry weights (table 5) indicate one noticeable variation: the weight

TABLE 2
BURPEE GOLDEN COSMOS

COMPLETE NUTRIENT (CONTROL)		COMPLETE NUTRIENT WITH					
		NAPHTHALENEACETIC ACID		NICOTINIC ACID		NICOTINIC ACID AND B ₁	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots

DRY WEIGHT (GM.) OF RANDOM ROWS OF TEN PLANTS EACH

37.7	9.8	43.2	11.3	52.0	12.7	55.4	14.4
57.5	13.5	54.3	16.5	53.5	14.3	50.8	13.6
56.9	16.5	53.9	13.5	49.9	12.8	57.3	15.3
52.0	14.2	57.0	16.7	49.2	16.1	59.6	14.7
57.3	16.7	55.5	13.9	58.0	14.8	58.4	14.2
52.8	12.7	53.0	13.5	51.2	14.0	56.5	14.8
56.2	13.4	44.8	12.2	57.3	15.9	46.4	13.3
52.2	14.4	51.6	16.3	56.8	14.1	56.8	15.1
51.8	16.6	49.4	16.7	55.7	14.0	54.4	12.8
50.9	14.4	60.8	14.3	55.6	16.0	55.8	15.0
63.8	15.3	51.8	13.1	62.7	17.0	63.5	17.5
59.0	15.3	52.2	13.5	49.5	13.8	40.0	10.0
39.6	12.2	38.8	11.0	44.6	11.0	50.6	13.0

AVERAGE DRY WEIGHT PER ROW OF TEN PLANTS

52.9	14.23	51.2	14.04	53.54	14.3	54.3	14.1
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AVERAGE DRY WEIGHT PER PLANT

5.29	1.423	5.12	1.404	5.354	1.43	5.43	1.41
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of the total marigold plant, and more definitely the weight of the tops of the plants receiving naphthaleneacetic acid (0.01 mg. per liter of nutrient), have less dry weight than the controls. This variance indicates that the weight lies above the calculated figure of significance at 5 per cent probability. This decreased dry weight illustrates the fact mentioned earlier, that although naphthaleneacetic acid is known to initiate root growth from stems, it may also retard growth as a result of toxic action and thus affect general plant growth.

TABLE 3
HARMONY MARIGOLD

COMPLETE NUTRIENT		COMPLETE NUTRIENT WITH					
		NAPHTHALENEACETIC ACID		NICOTINIC ACID		NICOTINIC ACID AND B ₁	
Tops	Roots	Tops	Roots	Tops	Roots	Tops	Roots
DRY WEIGHT (GM.) OF RANDOM ROWS OF TEN PLANTS EACH							
35.6	10.0	32.0	8.6	38.6	10.6	37.0	9.4
31.9	8.6	29.5	9.2	36.6	9.6	38.1	9.7
38.9	8.5	29.9	8.8	3.9	9.3	35.0	9.7
33.7	8.3	31.5	9.1	36.0	8.0	38.0	10.2
32.3	8.6	29.7	9.6	36.9	10.7	36.4	10.8
35.0	10.1	28.1	8.0	36.1	9.7	33.6	11.2
37.0	10.9	32.6	9.8	36.0	10.9	36.0	11.2
39.2	11.8	29.7	9.6	38.0	9.0	38.2	9.5
36.5	11.7	32.9	9.6	38.9	10.9	38.3	10.0
38.5	10.0	37.4	10.7	41.5	12.3	37.8	11.0
40.4	12.4	36.0	11.2	41.2	12.8	42.0	10.2
41.4	13.2	37.1	11.6	43.8	12.6	44.9	13.3
41.3	10.9	35.5	10.5	35.9	9.7	40.2	11.0
AVERAGE DRY WEIGHT PER ROW OF TEN PLANTS							
37.05	10.4	32.45	9.7	38.3	10.5	38.1	10.6
AVERAGE DRY WEIGHT PER PLANT							
3.705	1.04	3.245	0.97	3.83	1.05	3.81	1.06

TABLE 4

MEAN DRY WEIGHT IN GRAMS OF THIRTEEN ROWS OF COSMOS PLANTS USED AS CONTROLS COMPARED WITH SAME UNDER TREATMENTS INDICATED; ALSO ESTIMATED SIGNIFICANT DIFFERENCES IN WEIGHT BETWEEN CONTROLS AND TREATED PLANTS

	I CONTROL	II NAPHTHA- LENE- ACETIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND II)	III NICOTINIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND III)	IV NICOTINIC ACID AND B ₁	SIGNIFI- CANT* DIFFER- ENCE (I AND IV)
Tops.....	52.9	51.2	5.43	53.5	4.99	54.3	5.44
Roots.....	14.2	14.0	1.69	14.3	1.47	14.1	1.50
Total plant...	67.1	65.2	6.57	67.8	6.12	68.4	6.68

*Significant differences are weights above which, at probabilities of 5 per cent, mean differences may be considered statistically significant.

The dry weights of marigold plants supplied with nicotinic acid, and with nicotinic acid and vitamin B₁ combined, showed no increased growth response. Obviously, although vitamin B₁ and nicotinic acid are necessary for root growth, the supply produced by this plant is adequate for its needs. Consequently the external application of such substances, at least in the concentration used, has neither positive nor negative effect upon total plant growth.

TABLE 5
MEAN DRY WEIGHT IN GRAMS OF THIRTEEN ROWS OF MARIGOLD PLANTS USED AS CONTROLS COMPARED WITH SAME UNDER TREATMENTS INDICATED; ALSO ESTIMATED SIGNIFICANT DIFFERENCES IN WEIGHT BETWEEN CONTROLS AND TREATED PLANTS

	I CONTROL	II NAPHTHA- LENE- ACETIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND II)	III NICOTINIC ACID	SIGNIFI- CANT* DIFFER- ENCE (I AND III)	IV NICOTINIC ACID AND B ₁	SIGNIFI- CANT* DIFFER- ENCE (I AND IV)
Tops.....	37.0	32.4†	2.58	38.3	2.33	38.1	2.50
Roots.....	10.4	9.7	1.09	10.5	1.23	10.6	1.09
Total plant...	47.4	42.1†	3.48	48.8	3.36	48.7	3.30

* Significant differences are weights above which, at probabilities of 5 per cent, mean differences may be considered statistically significant.

† Weights indicating statistical significance.

Summary

1. Neither vitamin B₁ nor naphthaleneacetic acid supplied to chrysanthemum plants in a concentration of 0.1 mg. per liter of complete nutrient solution produced any apparent growth responses or significant difference in dry weight.
2. Chrysanthemum plants receiving naphthaleneacetic acid in a concentration of 0.1 mg. per liter of minus K nutrient solution showed an increase in dry weight over the controls, indicating that the acid can compensate to some extent for the lack of potassium.
3. No significant difference in dry weight occurred between cosmos plants used as controls and those receiving 0.01 mg. of naphthaleneacetic acid, nicotinic acid, or nicotinic acid and vitamin B₁ per liter of complete nutrient.
4. Marigold plants showed little variation in dry weight when supplied with nicotinic acid, or with a combination of nicotinic acid and vitamin B₁.
5. A significant decrease in total dry weight of the marigold plants supplied with naphthaleneacetic acid (0.01 mg. per liter) indicates a retarding effect of the acid on growth.
6. None of the treatments to which the cosmos and marigold plants were subjected resulted in visible effects upon their habit of growth or upon floral initiation.

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LITERATURE CITED

1. BONNER, JAMES, Nicotinic acid and the growth of isolated pea embryos. *Plant Physiol.* 13:865-868. 1939.
2. ———, On the growth factor requirements of isolated roots. *Amer. Jour. Bot.* 27:692-701. 1940.
3. BONNER, JAMES, and GREENE, JESSE, Further experiments on the relation of vitamin B₁ to the growth of green plants. *BOT. GAZ.* 101:491-499. 1939.
4. GRACE, N. H., Physiologic curve response to phytohormones by seeds, growing plants, cuttings, and lower plant forms. *Canad. Jour. Res. Sec. C.* 15:538-543. 1937.
5. GORHAM, P. R., Measurement of the response of *Lemna* to growth-promoting substances. *Amer. Jour. Bot.* 28:98-101. 1941.
6. HAMNER, C. L., Effects of vitamin B₁ upon the development of some flowering plants. *BOT. GAZ.* 102:156-168. 1940.
7. HAMNER, K. C., and KRAUS, E. J., Histological reactions of bean plants to growth promoting substances. *BOT. GAZ.* 98:735-807. 1937.
8. ROBBINS, W. J., and BARTLEY, MARY, Vitamin B₁ and the growth of excised tomato roots. *Science* 85:246-247. 1937.
9. ZIMMERMAN, P. W., and WILCOXON, FRANK, Several chemical growth substances which cause initiation of roots and other responses in plants. *Contrib. Boyce Thomp. Inst.* 7:209-228. 1935.

PHYSIOLOGICAL AND CHEMICAL RESPONSES OF BEAN AND TOMATO PLANTS TO ALPHA NAPHTHALENE ACETAMIDE AND PHENYLACETIC ACID¹

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Introduction

The investigations here reported deal with some of the physiological, chemical, and anatomical responses of bean and tomato plants to alpha naphthalene acetamide and phenylacetic acid when applied in nutrient solutions and as emulsion sprays to the tops of growing plants. Records have been made of the gross appearance of the plants, wet and dry weights, and content of total nitrogen, calcium, and phosphorus.

Red Kidney bean and Bonny Best tomato were used as the experimental plants. The general procedure for experiments I-IV was to plant four seeds in a 1½-liter glazed crock containing previously unused white quartz sand. Seven to nine days after planting, when the first primary leaves had expanded, the plants were thinned for uniformity to one and two per pot. The pots were arranged in rows of eight plants each on greenhouse benches. Each entire row was given specific treatment, and the treatments were randomized to permit statistical analyses of the harvested material.

The naphthalene acetamide used in the experiments was first dissolved in 10 cc. of 95 per cent ethyl alcohol and added to the specific quantity of nutrient solution required for each treatment just before watering. The first applications were made when the bean plants were thinned. The plants were harvested, each row separately, shortly after the first flowers appeared.

The concentrations of salts used in making the complete nutrient solution were: 0.0045 mol MgSO_4 , 0.0045 mol KH_2PO_4 , 0.0060 mol $\text{Ca}(\text{NO}_3)_2$, together with the minor elements. In experiments I, III, and IV lesser amounts of phosphate were used, for example, $\frac{1}{8}$ phosphate means that 0.00025 mol KH_2PO_4 was used instead of 0.0045 mol.

Chemical analyses were made of the plants used in experiment III. The calcium content was determined volumetrically (3). The Kjeldahl-Gunning method was used in determining the total nitrogen content (3). Phosphorus content was determined colorimetrically, using phosphorus molybdate in solution (3).

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² Agent, Bureau of Plant Industry, U.S. Department of Agriculture.

Experimentation

ALPHA NAPHTHALENE ACETAMIDE SUPPLIED IN NUTRIENT SOLUTION

EXPERIMENT I.—Bean seeds were planted May 2, 1941, and thinned to two per pot on May 8. Sixteen different combinations were run. Four concentrations of potassium phosphate in the nutrient solutions were used: complete, $\frac{1}{18}$ and $\frac{1}{36}$ as much phosphate as in the complete nutrient, and no phosphate. In combination with each of these nutrient solutions, a range of four concentrations of naphthalene acetamide— 10^{-5} , 10^{-7} , 10^{-9} , and 0—was used.

The plants were given nutrient solution every other day, with or without the addition of the naphthalene acetamide, the total number of applications containing acetamide throughout the experiment being six. The plants were harvested June 3, 1941, and wet and dry weights of tops and roots were measured. As a result of this experiment it was found that 10^{-5} concentration of naphthalene acetamide was the greatest amount that could be used effectively without resultant toxicity to the plants. The most effective range of concentration for increased root development appeared to be between 10^{-6} and 10^{-7} .

EXPERIMENT II.—This was performed to determine at what point within this range of naphthalene acetamide concentration (10^{-6} to 10^{-7}) the greatest root development would take place. For this purpose bean seeds were planted June 25 and thinned to one plant per pot on July 1. Eighteen replications of the following five concentrations of naphthalene acetamide were used in complete nutrient solution: 0, 10^{-7} , $10^{-6.67}$, $10^{-6.34}$, and 10^{-6} . Six applications of the solutions containing the naphthalene acetamide were made. On July 25 the plants were harvested and the wet and dry weights of tops, roots, and hypocotyls recorded (table 2).

EXPERIMENT III.—Two concentrations of phosphate were used. Nine different treatments were given and six replications of each treatment were run (table 1). The seeds were planted June 20, 1941, and the seedlings selected as in the first two experiments. The plants were harvested July 14. Wet weights of the tops and dry weights of tops, roots, and hypocotyls were recorded. Chemical analyses of total nitrogen, calcium, and phosphorus content were made of the tops, roots, and hypocotyls of the plants that had received treatment numbers 1-6 (tables 1, 3).

Plants grown without phosphorus, experiment I, showed no root stimulation, and those grown on a $\frac{1}{36}$ phosphate level showed only slight root stimulation when supplied with acetamide. All other levels of the phosphate concentration resulted in marked increase in root growth upon application of the acetamide in concentration of 10^{-6} . Top growth was inhibited when the phosphate concentration was $\frac{1}{18}$ or less.

From tables 1 and 2, experiment III, it is obvious that the concentration of naphthalene acetamide in the nutrient solution, and the number of applications, strikingly affect the growth response. One application resulted in a less weight of dry matter of tops of 23.29 gm. as compared with 28.70 gm. Root growth was slightly increased by one application, from 6.89 to 7.24 gm. In the case of root growth, the effects resulting from three applications were similar to those from six. This was not true for top growth.

The data in table 2, experiment II, show that a $10^{-6.67}$ concentration of naphthalene acetamide produced the greatest increase in amount of root growth. Growth of the tops was inhibited with increased concentration of acetamide within the range shown in table 2.

TABLE 1
EXPERIMENT III; BEAN PLANTS

TREATMENT NUMBER	NUMBER OF APPLI- CATIONS	CONCENTRATION OF POTASSIUM PHOSPHATE (MOLS)	CONCENTRA- TION OF NAPH- THALENE AC- ETAMIDE
1.....	0	0.000250	0
2.....	6	0.000250	10^{-6}
3.....	6	0.000250	$10^{-5.5}$
4.....	0	0.00150	0
5.....	6	0.00150	10^{-6}
6.....	6	0.00150	$10^{-5.5}$
7.....	1	0.00150	10^{-6}
8.....	2	0.00150	10^{-6}
9.....	3	0.00150	10^{-6}

The calcium contents of the plants grown in experiment III (table 3) showed a percentage dry weight increase in tops with the acetamide treatment. The greatest percentage increase occurred when the largest amount of acetamide was supplied. A similar trend was noted in the hypocotyls. The roots showed no relative increase in calcium content in the plants of the $\frac{1}{8}$ phosphate group and showed a relative decrease in the $\frac{3}{8}$ phosphate group, the decrease being from 23.34 mg./gm. to 15.63 mg./gm.

The percentage of calcium content of the control tops on $\frac{3}{8}$ phosphate was approximately the same as that of the control roots also on $\frac{3}{8}$ phosphate. The calcium content of the plants on the $\frac{3}{8}$ phosphate nutrient solution that contained $10^{-5.5}$ acetamide showed a greater difference between tops and roots than did those receiving no acetamide, the content being 41.10 mg./gm. for the tops and 15.63 mg./gm. for the roots.

The phosphorus content increased in the tops of the plants treated with concentrations of 10^{-6} and $10^{-5.5}$ naphthalene acetamide. In the $\frac{3}{8}$ phosphate group

TABLE 2

DRY WEIGHT (IN GRAMS) OF TOPS, ROOTS, AND HYPOCOTYLS OF BEAN PLANTS TREATED WITH VARYING CONCENTRATIONS OF ALPHA NAPHTHALENE ACETAMIDE IN SOLUTIONS CONTAINING VARIOUS AMOUNTS OF KH_2PO_4

KH_2PO_4 (MOLS)	CONCENTRA- TION OF ALPHA NAPHTHALENE ACETAMIDE	DRY WEIGHT (GM.)		
		TOPS	ROOTS	HYPOCOTYLS

EXPERIMENT I

0.....	0	7.91	4.74
0.....	10^{-9}	7.98	4.64
0.....	10^{-7}	7.28	4.54
0.....	10^{-5}	2.01	1.97
0.000125.....	0	13.20	6.24
0.000125.....	10^{-9}	14.31	7.01
0.000125.....	10^{-7}	12.63	6.54
0.000125.....	10^{-5}	3.13	3.47
0.000250.....	0	20.30	7.90
0.000250.....	10^{-9}	17.91	7.90
0.000250.....	10^{-7}	15.58	8.87
0.000250.....	10^{-5}	3.50	3.74
0.0045.....	0	28.55	7.90
0.0045.....	10^{-9}	28.67	7.80
0.0045.....	10^{-7}	26.30	8.70
0.0045.....	10^{-5}	3.93	3.67

EXPERIMENT II

0.0045.....	0	28.3	6.52	2.07
0.0045.....	10^{-7}	25.7	7.77	1.87
0.0045.....	$10^{-6.67}$	22.2	8.90	1.63
0.0045.....	$10^{-6.34}$	19.1	8.63	1.50
0.0045.....	10^{-6}	15.9	7.56	1.20

EXPERIMENT III

0.00025.....	0	22.68	7.11	2.43
0.00025.....	10^{-6}	16.13	8.91	1.75
0.00025.....	$10^{-5.5}$	8.70	8.28	1.78
0.0015.....	0	28.70	6.80	2.12
0.0015.....	10^{-6}	17.83	8.57	1.53
0.0015.....	$10^{-5.5}$	7.78	7.65	1.37
0.0015.....	10^{-6*}	23.29	7.24	2.05
0.0015.....	$10^{-6†}$	20.90	7.70	2.16
0.0015.....	$10^{-6‡}$	21.70	8.34	2.08

* One application. † Two applications. ‡ Three applications.

the control tops showed a phosphorus content of 3.84 mg./gm. In the same phosphate group the tops of the plants given the acetamide in concentration of 10^{-6} contained 4.19 mg./gm. phosphorus, while those treated with a $10^{-5.5}$ solution contained 6.40 mg./gm. The plants that had received the $\frac{3}{8}$ phosphate nutrient solution showed a much higher phosphorus content in the tops and roots than those that had received the $\frac{1}{8}$ phosphate solution. The difference between the phosphorus contents of the tops and of the roots increased with increasing amounts of the acetamide supplied.

TABLE 3

EXPERIMENT III; BEAN PLANTS

CALCIUM, PHOSPHORUS, AND TOTAL NITROGEN CONTENT OF PLANTS TREATED WITH AND WITHOUT ALPHA NAPHTHALENE ACETAMIDE IN NUTRIENT SOLUTION
WEIGHT IN MG. PER GM.

SUBSTANCE DETER- MINED	PLANT PART	CONCENTRATION OF ALPHA NAPHTHALENE ACETAMIDE IN NUTRIENT SOLUTION					
		0.00025 MOL KH_2PO_4			0.0015 MOL KH_2PO_4		
		0	10^{-6}	$10^{-5.5}$	0	10^{-6}	$10^{-5.5}$
Ca	Tops.....	23.37	28.60	38.94	23.67	29.98	41.10
	Roots.....	14.21	14.29	13.03	23.34	17.16	15.63
	Hypocotyls.....	9.09	11.57	11.97	8.58	10.67	12.57
P	Tops.....	1.80	2.11	2.40	3.84	4.19	6.40
	Roots.....	0.84	0.89	0.94	2.20	2.15	2.20
	Hypocotyls.....	0.93	1.08	1.48	2.22	2.58	3.07
Total ni- trogen	Tops.....	39.66	42.73	44.50	38.96	40.44	40.18
	Roots.....	30.79	35.88	35.90	28.67	33.47	38.01
	Hypocotyls.....	16.55	17.78	20.85	14.83	15.43	23.60

The total nitrogen content was greater in the tops, roots, and hypocotyls of all plants treated with acetamide. In the $\frac{1}{8}$ phosphate group the total nitrogen content for the tops showed a progressive percentage increase with increase in concentration of acetamide. The roots of the plants that had received acetamide in concentrations of 10^{-6} showed a marked percentage increase in nitrogen content over the controls, whereas those that had received $10^{-5.5}$ acetamide showed only a slight increase over those having received 10^{-6} . In the $\frac{3}{8}$ phosphate group there was a progressive percentage increase in total nitrogen in the roots with increasing concentration of acetamide. The tops in this group showed an increase with acetamide treatment but displayed no great differences due to the different concentrations. The hypocotyls in both groups showed marked percentage increase with increasing acetamide concentration.

Histological sections of the roots of bean plants showed the following differences between the plants treated with a concentration of naphthalene acetamide of 10^{-6} and the controls. The number of secondary roots of the treated plants was greater than those of the untreated, although many of these roots were short and stubby. The diameter of the younger roots of the treated plants was approximately twice as great as that of the controls. The cells of the embryonic region of the treated roots showed much denser cytoplasm. The subepidermal cortical cells showed many more radial divisions of the cells as contrasted with the tangential divisions in the controls. A number of the cortical cells were enlarged and spherical, resulting in many air spaces. The most striking feature of the treated plants was the very early maturation of the elements of the xylem and phloem. Maturation in most of the roots took place within less than 0.6 mm. of the apical meristem. Division of the cells of the pericycle over the xylem points was less pronounced in the controls than in the treated plants. In the treated plants pericyclic activity was most pronounced back from the apical meristem, where the xylem was nearly mature. At this level there was a bulbous enlargement of the root. In a few of the treated roots radial division of the epidermal cells occurred. In some of the roots of the treated plants no differences from the controls could be observed.

ALPHA NAPHTHALENE ACETAMIDE APPLIED AS EMULSION SPRAY
TO THE LEAVES

EXPERIMENT IV.—On April 29, 1941, four seeds of bean were planted in each of 1200 glazed crocks in clean unused quartz sand. Immediately after planting, the pots were divided into three lots, one lot receiving complete nutrient solution, another a similar solution containing only $\frac{3}{8}$ as much phosphorus, and the third lot containing $\frac{1}{8}$ as much phosphorus. The pots were then completely randomized in rows on four greenhouse benches, there being twenty-one replications of each treatment. On May 6 the number of plants per pot was reduced to one. On May 7 the primary leaves had completely unfolded but were not of full size. The epicotyl had not yet begun to elongate. On the afternoon of this day, half the plants of each lot were sprayed with an emulsion of naphthalene acetamide in the amount of 400 mg./l. They were given a similar spraying on May 15 and were watered with the respective nutrient solutions on May 14, 19, and 22, and with distilled water on May 17.

All the plants grew well. The tops of those sprayed with the acetamide emulsion were not so large as those left unsprayed. Very little difference in growth was noted between the plants supplied with the different quantities of phosphorus in the nutrient medium. On May 30 the tops, and on June 2 the roots, were harvested. Each individual row was kept separate and the dry weights determined. A summary of the results is given in table 4.

PHENYLACETIC ACID SUPPLIED IN NUTRIENT SOLUTION TO BEAN
AND TOMATO PLANTS

EXPERIMENT V.—On April 14, four seeds of bean were planted in each of a number of glazed crocks containing unused white quartz sand and then sprinkled

TABLE 4
EXPERIMENT IV; BEAN PLANTS

	COMPLETE				$\frac{3}{8}$ PHOSPHORUS				$\frac{1}{18}$ PHOSPHORUS			
	TOPS		ROOTS		TOPS		ROOTS		TOPS		ROOTS	
	CON- TROL	SPRAYED	CON- TROL	SPRAYED	CON- TROL	SPRAYED	CON- TROL	SPRAYED	CON- TROL	SPRAYED	CON- TROL	SPRAYED
	DRY WEIGHTS OF TOPS AND ROOTS PER 8 PLANTS (IN GRAMS)											
Weight.....	24.4	22.3	10.1	10.1	25.2	22.9	11.1	11.6	24.4	22.2	10.3	10.4
	CALCIUM AND PHOSPHORUS CONTENT OF TOP LEAVES AND STEMS (IN MG./GM.)											
Ca.....	12.3	12.4	8.2	8.3	15.4	14.1	13.3	11.4	17.9	16.6	14.8	11.2
P.....	5.5	6.4	3.3	3.3	4.8	5.0	3.4	3.4	2.4	2.5	1.4	1.4

TABLE 5
EFFECT OF PHENYLACETIC ACID SUPPLIED IN NU-
TRIENT SOLUTION UPON TOPS AND ROOTS OF BEAN
PLANTS, EXPRESSED IN GRAMS PER 10 LOTS OF
PLANTS

CONCENTRATION	TOPS	ROOTS
10^{-6}	36.8	14.3
10^{-7}	36.7	14.3
10^{-8}	37.4	14.8
10^{-9}	37.0	15.2
10^{-10}	36.8	14.7
10^{-11}	37.3	14.6
10^{-12}	37.8	16.0
Control.....	39.2	15.8

with tap water. On April 21 the plants were thinned to two per crock and divided into ten lots of eighty crocks each. These lots were arranged in sequence on four greenhouse benches. Each lot was subdivided into eight rows of ten plants each. The rows in each lot were randomized by drawing the numbers according to chance. Within each lot one row served as a control; the others received phenyl-

acetic acid in complete nutrient solution as follows: 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11} , 10^{-12} . Waterings with the respective solutions were made on April 22, 25, 29, and May 2, 6, and 9. All were given one watering with distilled water on May

TABLE 6
EXPERIMENT VI; TOMATO PLANTS
DRY WEIGHT OF TOPS AND ROOTS (IN GRAMS) ADDED TO OBTAIN DRY WEIGHT OF TOTAL
PLANT. STATISTICAL ANALYSIS MADE ON BASIS OF DRY
WEIGHT OF TOTAL PLANT; JULY, 1941

Row	CONCENTRATION OF PHENYLACETIC ACID IN NUTRIENT SOLUTION							
	CONTROL	10^{-12}	10^{-11}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Tops								
1.....	23.1	23.2	25.2	25.3	23.6	25.0	24.7	26.2
2.....	21.6	23.7	26.2	24.6	24.5	25.2	24.8	24.7
3.....	23.8	25.3	26.3	25.3	24.6	26.7	26.6	27.2
4.....	22.9	26.2	24.4	26.7	24.4	25.2	24.9	22.4
5.....	22.7	26.9	24.5	25.4	24.1	26.3	27.3	25.4
6.....	20.3	26.0	23.6	26.0	26.8	27.6	25.5	24.6
7.....	22.3	23.8	25.6	22.6	24.2	22.6	22.9	22.7
8.....	22.2	24.1	23.3	24.0	22.4	23.7	24.9	24.0
9.....	19.4	23.5	23.6	28.1	23.7	26.2	21.7	24.8
10.....	20.3	26.1	25.0	25.0	22.6	20.9	29.4	22.4
11.....	20.4	23.6	22.5	22.1	24.2	22.0	22.8	23.5
12.....	21.1	23.0	23.0	23.8	26.4	24.8	22.7	24.8
Sum.....	260.1	295.4	293.2	298.9	291.5	296.2	298.2	292.7
Average.....	21.7	24.6	24.4	24.9	24.3	24.7	24.9	24.4
Roots								
1.....	12.1	11.5	9.1	11.2	10.5	11.2	10.4	10.8
2.....	10.7	11.5	10.5	10.8	15.3	11.3	9.3	9.1
3.....	13.8	12.0	11.3	11.3	10.5	11.6	11.7	10.3
4.....	11.2	12.7	9.2	10.8	10.9	14.3	11.2	13.8
5.....	10.0	12.1	13.8	11.6	11.8	11.1	12.5	10.4
6.....	11.2	13.0	10.4	9.6	13.6	12.4	11.3	10.4
7.....	9.6	9.2	12.5	10.1	11.1	8.7	8.8	8.7
8.....	10.4	9.2	9.8	11.7	9.4	11.3	12.7	10.7
9.....	8.4	9.4	8.2	13.1	9.4	10.8	8.4	9.3
10.....	11.1	13.0	12.1	11.8	10.5	12.5	15.6	9.6
11.....	8.7	9.8	12.0	13.1	10.8	10.1	10.4	9.2
12.....	10.0	8.5	9.5	9.8	11.7	10.2	10.7	11.2
Sum.....	127.2	131.9	128.4	134.9	135.5	135.5	133.0	123.5
Average.....	10.6	11.0	10.7	11.2	11.3	11.3	11.1	10.3

11. The tops and roots of each row were harvested separately on May 12, and their dry weights were determined. Table 5 shows a summary of results, analysis of which shows no significant differences owing to the treatments.

EXPERIMENTS VI, VII, VIII.—Tomato plants were used in three experiments in which phenylacetic acid was used in the nutrient solution. Experiments VI and VII were conducted at Beltsville, Maryland, and experiment VIII at Chicago, Illinois. Tomato seeds were planted in quartz sand and watered with the complete nutrient solution as previously described. The first application containing phenylacetic acid was made when the plants were 3-4 inches in height, and subsequently two or three times during each succeeding week. The phenylacetic acid was first dissolved in ethyl alcohol and then added to the nutrient solution. The concentration ranged from 1 part in 10,000 to 1 part in 1,000,000,000,000. Nine

TABLE 7

DRY WEIGHT OF TOPS AND ROOTS OF TOMATO PLANTS UNDER VARIOUS TREATMENTS
OF EXPERIMENTS VI, VII, AND VIII, EXPRESSED IN
GRAMS DRY WEIGHT PER 8 PLANTS

CONCENTRATION OF PHENYLACETIC ACID	EXPERIMENT VI		EXPERIMENT VII		EXPERIMENT VIII	
	Tops	Roots	Tops	Roots	Tops	Roots
10^{-4}	43.5	11.0
10^{-5}	24.4	10.3	48.2	12.0
10^{-6}	24.9	11.1	97.9	29.0	50.4	12.1
10^{-7}	24.7	11.3	51.1	12.2
10^{-8}	24.3	11.3	92.8	27.5	53.5	12.05
10^{-9}	24.9	11.2	51.0	12.1
10^{-10}	24.4	10.7
10^{-11}	24.6	11.0
10^{-12}	94.5	29.5
0.....	21.7	10.6	95.0	27.8	51.5	12.1
0*.....	96.1	27.1

* Plus alcohol.

to twelve replications of each treatment were made, using eight crocks as a unit. The plants were harvested at the time of their first blooming. Statistical analyses were made of the total dry weight of the plants.

For experiment VI, tomato plants 3-4 inches high received their first treatment on June 30. Eight different treatments were used, and twelve replications of each were made. The concentration of the phenylacetic acid ranged from 10^{-6} to 10^{-12} . A control to which no acid was added was used for comparison. A 200-cc. solution was added to each pot containing one plant, two or three times a week. The plants were harvested when the first blossom cluster opened, on July 30. All plants receiving treatments with the acid showed approximately a 15 per cent increase in top growth over that of the controls and approximately a 7 per cent increase in root growth. The results are given in table 6, which is representative of the data taken in all three experiments.

Experiment VII, also run at Beltsville, was conducted to determine whether or not greater dilutions of phenylacetic acid would result in increased growth. More favorable weather conditions prevailed during the course of this experiment than in the case of experiment VI, so that the plants grew larger. The tomato plants received their first treatment August 16. The acid was applied in concentrations of 10^{-6} , 10^{-8} , and 10^{-12} . Two controls were used, one containing nutrient solution alone, the other containing nutrient solution plus the amount of alcohol used as a carrier for the acid. The plants were harvested when the third blossom cluster opened, on September 30. Dry weights of tops and roots were determined. No significant differences between the plants receiving the various treatments were obtained in this experiment (table 7).

Experiment VIII, carried on in Chicago, ran concurrently with experiment VI. The concentration range of phenylacetic acid used was from 10^{-4} to 10^{-9} . The plants received their first application August 14. Three treatments per week were administered. The plants were harvested September 23. The stronger concentrations, 10^{-4} and 10^{-5} , produced a decrease in both top and root growth. A slight increase in top growth (approximately 5 per cent) was obtained as a result of treatments with the acid in the concentration of 10^{-9} .

Discussion

MITCHELL and STEWART (5) found that when alpha naphthalene acetamide was applied in high concentrations as an emulsion spray to the tops of bean plants, increased root growth and decreased top growth resulted. When applied in low concentrations, top growth was increased. When applied in lanolin paste to decapitated bean plants, mobilization of solid materials toward the treated region resulted. KRAUS and MITCHELL (1), in a later experiment, found that spraying the tops with an emulsion of alpha naphthalene acetamide also resulted in increase of the amount of cell-wall thickening and development of secondary xylem in the stem and petioles.

MITCHELL (4) reported that when a lanolin mixture of alpha naphthalene acetamide was applied to the stem of bean plants the percentage of starch, dextrin, and sugar in the roots, hypocotyls, and first internode was greater than in control plants. He also reported an inhibition of the translocation of carbohydrates from the leaves. LAUDE (2) reported increased root growth of bean when low concentrations of naphthalene acetamide were supplied in the nutrient solution.

In the present investigation, when naphthalene acetamide was supplied in the nutrient solution, the plants reacted in a manner somewhat similar to those to which it had been supplied as a spray or in a lanolin paste. Leaf expansion was inhibited and curling and thickening of the leaf blade was noticeable. Relatively less growth of the tops was one of the most striking reactions. Root growth was

greater than that of the controls by as much as 37 per cent in some cases. The percentage of calcium, phosphorus, and nitrogen in the tops was greatly increased, although the absolute amount was less because of the less amount of total growth made by the treated plants.

The increased root growth might be explained by the diversion of carbohydrates to the roots, which may in part account for the relative percentage increase of calcium, phosphorus, and nitrogen and their accumulation in the tops. The increased ash content of the tops of the treated plants is correlated with decreased top growth. The greater root growth was accompanied either by no change in calcium and phosphorus of the root or by a percentage decrease in both of them. Thus the percentage of calcium and phosphorus in the plant tissue is apparently negatively correlated with the total dry weight.

In the control plants grown with an adequate supply of all the minerals, the percentages of calcium in the tops and in the roots were approximately the same, while in some of the plants treated with the acetamide the calcium content was two and one-half times as great in the tops as in the roots. Compared with their respective controls, there was a much wider difference between the calcium and phosphorus contents of the tops than of the roots on the basis of increased content of acetamide in the nutrient solution. The ratio of the calcium of the tops to that of the roots of the untreated plants was 1:1, while that of the treated plants was 2.5:1. The ratio of top to root growth of the control plants was 4:1, while that of the treated plants was 1:1. There would appear to be an inverse relationship between the top-root ratio of dry matter and the top-root ratio of calcium content.

As reported by MITCHELL and STEWART (5), increased top growth resulted from spraying the tops of bean plants with dilute emulsions of naphthalene acetamide; in the experiments reported here in no instance was an increase obtained when this substance was supplied in the nutrient solution. Greatest increase of root growth resulted when a concentration of 0.34 mg./l. of naphthalene acetamide was supplied in the nutrient solution; increase of root growth, as reported by MITCHELL and STEWART, resulted when 625 mg./l. was used as an emulsion spray.

When alpha naphthalene acetamide was applied to the tops in the form of a spray in a manner similar to that reported by MITCHELL and STEWART (except that the plants were allowed to grow much longer after the initial treatment), the plants showed a slight depression of top growth and dry weight and no differences in dry weight of the roots. No increase was found in the content of the uppermost leaves or stems owing to the spray applied. There was, however, greater content of calcium in the tops in those cultures where there was less phosphorus in the nutrient solution; but this difference was independent of any of the spraying treatments.

Summary

1. Alpha naphthalene acetamide when applied in the nutrient solution results in less top growth and increased root growth of Red Kidney bean plant.

2. Relative percentage contents of calcium, phosphorus, and nitrogen in the bean plant are increased by the presence of naphthalene acetamide in the nutrient solution.

3. The xylem and phloem mature earlier and are more extensive in the roots of bean plants treated with naphthalene acetamide in the nutrient solution.

4. Spraying the tops of bean plants with naphthalene acetamide did not result in increase of calcium or phosphorus content in the top leaves or stems, or result in increased dry weight of the plants.

5. Phenylacetic acid when supplied in the nutrient solution to bean plants did not significantly increase the total dry weight.

6. Phenylacetic acid when supplied in the nutrient solution to Bonny Best tomato may increase top and root growth in young plants. The evidence, however, is not conclusive.

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LITERATURE CITED

1. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. *BOT. GAZ.* 101:204-221. 1939.
2. LAUDE, H. M., Combined effects of potassium supply and growth substances on plant development. *BOT. GAZ.* 103:155-167. 1941.
3. METHODS OF ANALYSIS. A.O.A.C. 4th ed. 1935.
4. MITCHELL, J. W., Effect of naphthalene acetic and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plants. *BOT. GAZ.* 101:688-699. 1940.
5. MITCHELL, J. W., and STEWART, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. *BOT. GAZ.* 101:410-427. 1939.

POLARITY OF AUXIN TRANSPORT IN INVERTED TAGETES CUTTINGS¹

F. W. WENT

(WITH ONE FIGURE)

Ever since the early work on regeneration of roots and outgrowth of buds on cuttings, the polarity of this phenomenon has been stressed. Notable exceptions to this behavior were found only in branches with preformed root primordia. More recently it was shown that a reversal in the polarity of root formation could also be achieved by applying high concentrations of auxins or auxin-like substances. Then roots appear at the place of application regardless of the original polarity of the plant.

This polarity in organ formation was demonstrably due to polar auxin transport within the branch. It had not been possible to reverse the polarity of auxin transport in the living cell (1), and the apparent lack of polarity in certain experiments could be attributed to auxin leakage through nonliving tissues (3) or along wet surfaces (7). For these reasons it seemed of interest to study the polarity of auxin transport in stem pieces which were functioning in a polarly inverted direction.

The question was: If a piece of stem has produced shoots near its original base which are supplied with water and salts through roots which developed near its original apex, in which direction will the auxin then move through the stem? For it is clear that in this case water, salt, and organic food transport are inverted.

To produce such stem pieces with inverted food transport, cuttings of *Tagetes* were made so as to include 2-3 nodes. They were placed either with their base or with their apex in auxin solution and then planted in sand. Figure 1 shows the position of the cuttings. In this figure and throughout the following discussion, apex and base will be used to designate the original or morphological apex and base of the stem piece, irrespective of its position toward gravity. In both cases roots developed at the treated region of the cutting. The axillary buds on the middle nodes were removed, and thus in the upright cuttings only the buds near the apex grew out, and in the inverted cuttings only those at the original base grew out. This left a portion of stem approximately 10 cm. long between roots and growing lateral shoots, which was ample for auxin transport determination.

The first inverted cuttings tested were kindly supplied from the greenhouses at Beltsville by Dr. E. J. KRAUS. Although the cuttings were not in the best condi-

¹ Report of work undertaken as part of a cooperative project between the California Institute of Technology and the Bureau of Plant Industry, U.S.D.A.

tion upon arrival in Pasadena, the following results with auxin transport were obtained. Stem sections 6–8 mm. long were cut from the inverted stems (fig. 1, 2) and from the normal lateral shoots (fig. 1, 3). They were each placed on a 1-mm. thick agar block 5×5 mm. An agar block of the same dimensions containing 10 mg./l. or 50 mg./l. indoleacetic acid was placed on top of the section, and 2 hours later the bottom blocks were analyzed (after cutting them into four pieces)

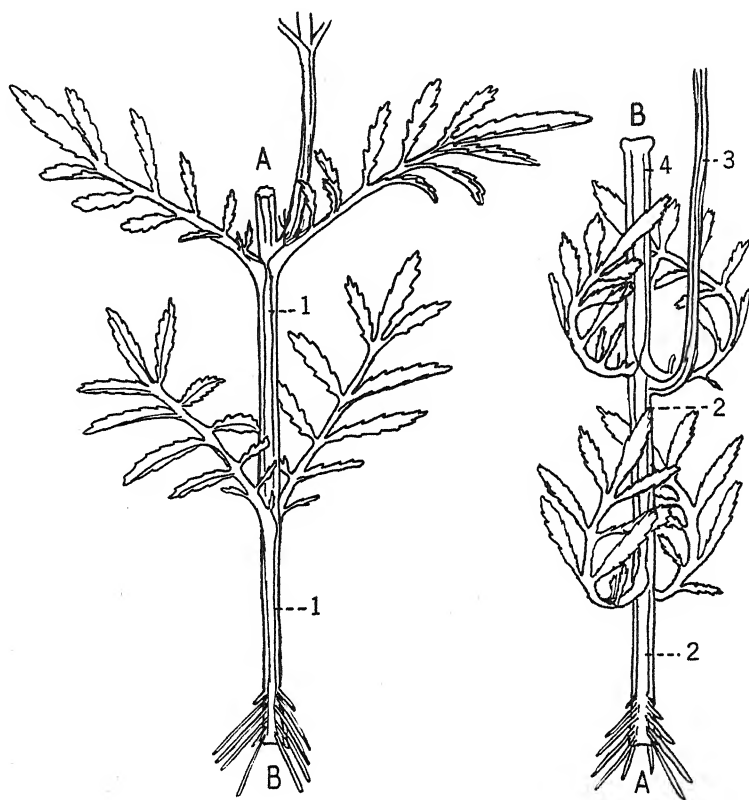


FIG. 1

on four standard *Avena* test plants each. In eight stem pieces from lateral shoots in which normal apex-to-base auxin transport was tested, three clearly showed transport. In eight pieces with inverse transport all tests were negative. It can be concluded that the lateral shoots on these inverted cuttings have normal polar auxin transport. In nine sections from the inverted part of the cutting, in which auxin moved from original apex to base, five gave transport out of nine. Of nine sections with auxin applied at the base, seven gave strong transport. Thus the polarity in auxin transport had disappeared in the inverted stem portion of the cutting.

In a subsequent set of tomato and *Tagetes* cuttings, made in winter, the original polarity was still much in evidence in the inverted cuttings, and only a few indications of inverted auxin transport were found.

A conclusive set of auxin transport determinations was carried out on *Tagetes* cuttings made July 25, 1939. All cuttings had two nodes and were treated, either at the base or the apex, with 100 mg./l. indoleacetic acid for 18 hours. They were potted on August 2, when all had formed large numbers of roots at the treated end. In table 1 all auxin transport determinations with these cuttings have been compiled. In most cases two rows of twelve test plants each were used to determine the amount of auxin transported through the 6-8 mm. stem sections. The values of both test rows are included in the table. It will be seen that there is considerable variability in the auxin transport, but that qualitatively the figures leave no room for doubt. In the main stem of normal upright cuttings auxin moves readily from apex to base, but (except in one case, presumably an experimental error due to inversion of the stem section) no auxin is transported from base toward the apex. In the inverted cuttings the inverted stem between roots and insertion of the lateral branch (fig. 1, 2) has a different behavior. Immediately after inverting the cuttings the polarity was still normal. After lateral shoots were well developed, 21 days after inversion, this original polarity was still very pronounced, but small amounts of auxin seem to move already in base-to-apex direction.

Two, 3, and 6 weeks later, a strong auxin transport, almost as great as in the apex-to-base direction, was found in the inverse direction. There is no doubt about this effect, which agrees with that found in the preliminary determinations. It is interesting that the polarity as such is not reversed, but that the auxin transport in the original direction continues at about the same rate and that a second auxin transport in the opposite direction is added. This is brought about by the inverse position of roots and shoots in regard to the old stem, and it is not a function of the effect of gravity. This is indicated by the auxin transport through the inverted stem portion basal from the lateral shoots (4 in fig. 1 and table 1). With both roots and shoots inserted apically no inverse auxin transport developed, nor was there any disturbance of polarity in the lateral shoots of inverted cuttings.

This physiological polarity is in perfect agreement with previous views, especially by VÖCHTING, concerning morphological polarity of plant organs and tissues. VÖCHTING (4) had concluded from anatomical evidence that every living cell has a polar structure, and that the morphological and physiological polarity of organs is due to the polarity of their cells. Especially convincing was the evidence in his experiments with inversely implanted pieces of *Beta vulgaris* roots. Although in many cases junction of the tissues of host and graft occurred, irregular overgrowth indicated that they differed from normally implanted grafts. Anatomical investigation showed that where two apical or two basal ends of tissues were in

juxtaposition, no direct vascular connections between them were established. But always the cells growing out from both apical or both basal ends seemed to repulse each other, bending around in a half circle, so that ultimately when the cells from graft and host joined they had the correct polar position of base adjoining apex. A polar reversal of the implanted tissue was never observed, nor a base-to-base junction of cells, so that the polarity of the cell was also absolute. Later VÖCHTING (5) drew the same conclusions from grafting and regeneration in *Brassica oleracea*

TABLE 1

AMOUNT OF AUXIN (IN DEGREES STANDARD AVENA TEST CURVATURES) COLLECTED AT ONE END OF STEM SECTIONS OF TAGETES CUTTINGS 6-8 MM. LONG, WHEN 50 MG./L. INDOLEACETIC ACID IS APPLIED AT OTHER END. EACH VALUE IS MEAN OF AT LEAST 12 TEST PLANTS. NUMBERS IN BRACKETS REFER TO STEM PORTIONS OF FIGURE 1 (A-B, APEX TO BASE TRANSPORT; B-A, BASE TO APEX TRANSPORT)

DAYS BE- TWEEN MAKING CUTTINGS AND AUXIN TRANSPORT TEST	NORMAL UPRIGHT CUTTINGS (1)						INVERSE CUTTINGS (2)						LATERAL SHOOTS ON IN- VERSE CUTTINGS (3)	INVERSE CUTTING: PORTION OF MAIN STEM BASAL OF LATERAL SHOOTS (4)				
	APEX TO BASE TRANSPORT			BASE TO APEX TRANSPORT			APEX TO BASE TRANSPORT			BASE TO APEX TRANSPORT								
	A-B		MEAN	B-A		MEAN	A-B		MEAN	B-A		MEAN			A-B	B-A	A-B	B-A
15.....			12.4			0.5												
21.....	17.6	14.2	15.9	0.7	0.3	0.5	11.6	7.9	9.7	1.1	2.7	1.9						
36.....	13.7	11.7	12.7	0.7	1.1	0.9	12.1	16.6	14.3	7.7	11.6	9.6						
43.....	13.8	19.9	16.8	0.6	(11.0)	0.6	16.7	12.5	14.6	5.8	11.4	8.6						
66.....	15.2	18.1	16.6	0.2	0.3	0.2	31.4	27.0	29.2	6.0	18.3	12.1	21.5	0.5	26.0	0.5		
Mean...			14.9			0.5			16.9			8.0	21.5	0.5	26.0	0.5		

tubers. Development of inverted cuttings of *Salix* (6), rooted at their apex, confirmed the earlier results. In the beginning, growth of these cuttings was slow, but in many the original inhibition was overcome in the course of years. Then a large overgrowth had developed on such a cutting. The polarity of the cells in this overgrowth was irregular, but finally—where this overgrowth touched the thickened adventitious root—a continuous series of cells with base-to-apex junctions joined shoot with root. Thus in addition to the inverted tissues of the original stem, new tissues with the opposite polarity had developed. In an extensive anatomical study, NEEFF (2) also found that with inversion of function in stems and roots a reorientation of cells occurs, which finds its origin in form changes of the cambial cells. They bend around, and the new vascular tissue derived from these reversed cambial cells has also an inverted morphological polarity.

These cases give a probable explanation of the behavior of polar auxin transport in inverted *Tagetes* cuttings. The mature cells of the stem all retain their original polarity; therefore auxin transport in the basal direction remains unchanged. In line with VÖCHTING's and NEEFF's results, it has to be assumed that the outgrowth of buds basally from the roots results in the laying down of new tissues or perhaps new vascular bundles. In these new vascular elements, connecting root and shoot, the cells have the opposite polarity for auxin transport. As a result the gradual development of an auxin transport from original base to apex would be expected, not interfering with the existing polar mechanism and starting some time after rooting of the inverted cutting. It is likely that such a new vascular system with opposite physiological polarity could be observed anatomically. This still remains to be done. With this explanation as a good possibility, there is no certain basis as yet for assuming that the polarity of auxin transport in a cell can be inverted, even though the organ in which the cell occurs may be functioning in an inverted position.

Summary

In inverted *Tagetes* cuttings, which have developed roots at their original apex and shoots near their base, the original polar auxin transport persists, but in addition a new auxin transport from the original base toward the apex develops. The first indication of this inverse transport occurs 3 weeks after starting the inverted cutting, and after a month both normal and inverse auxin transports occur simultaneously in the inversely functioning portion of the stem.

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LITERATURE CITED

1. CLARK, W. G., Electrical polarity and auxin transport. *Plant Physiol.* 13:529-552. 1938.
2. NEEFF, F., Über polares Wachstum von Pflanzenzellen. *Jahrb. wiss. Bot.* 61:205-283. 1922.
3. SKOOG, F., Absorption and translocation of auxin. *Amer. Jour. Bot.* 25:361-372. 1938.
4. VÖCHTING, H., Über Transplantation am Pflanzenkörper. *Tübingen.* 1892.
5. ———, Untersuchungen zur experimentellen Anatomie des Pflanzenkörpers. *Tübingen.* 1908.
6. ———, Untersuchungen zur experimentellen Anatomie und Pathologie des Pflanzenkörpers. Bd. 2. *Tübingen.* 1918.
7. WENT, F. W., and WHITE, R., Experiments on the transport of auxins. *BOT. GAZ.* 100:465-484. 1939.

SYNTHETICALLY PRODUCED SUBSTANCE B

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Introduction

NIELSEN and HARTELIUS (2) succeeded in forming substance B by chemical means. Their first results were obtained by autoclaving the "*Rhizopus*-nutrient-solution" in the presence of filter paper and assaying by dry weight yields of *Aspergillus niger*. They also showed that the mineral salts played no part, while the important factors were the nitrogen source, the carbon source, and the filter paper. Various nitrogen sources were tried, and the results indicated that all organic ammonium salts were effective, that NH_4Cl had no effect, and that NaNO_3 produced an inhibiting factor. Tests with various carbon sources showed that all were more or less effective. Six different filter papers were tried, and Schleicher and Schull no. 597 proved to be the most effective. They also state that the ash of the filter paper gave results similar to those of the filter paper itself. This synthesized substance not only functioned similarly to substance B of the *Rhizopus* filtrate, but also—like the latter—it was soluble in water, insoluble in ether, and stable to oxidation by perhydrol.

In a subsequent paper (3), these workers demonstrated the production of this active substance by autoclaving 2 per cent lactic acid and 2 per cent glucose, at 135°C ., with filter paper or products obtainable from it. These last in decreasing order of effectiveness are: filter paper, ash of filter paper, HCl-soluble substances. NIELSEN and HARTELIUS also produced this active factor by autoclaving glucose and ammonium tartrate in the presence of ZnSO_4 . Their ultimate conclusions were: (a) filter paper has little or no effect on the growth of *Asp. niger*, but serves with substance B as a co-factor; (b) substance B can be formed chemically by autoclaving a nutrient carbon source and an ammonium salt in the presence of ZnSO_4 , filter paper, or various fractions of the latter.

DAGYS (1) obtained similar results. He showed that autoclaving malic acid, or one of its salts, and fructose in the presence of filter paper resulted in the formation of a substance (substance B?) effective with *Asp. niger*. He likewise showed that the ash of birch sap would adequately substitute for the co-factor of the filter paper. He, like NIELSEN and HARTELIUS, demonstrated that the physico-chemical properties of the synthetic substance B were similar to the one produced metabolically by *Rhiz. suinus*.

Several series of experiments were arranged to retest the results of the investigators just referred to, at the same time using another organism, *Rhiz. suinus*, and

with the hope of obtaining a better insight into the processes taking place. Any methods or procedures not outlined in detail below have been so treated in a previous section of this report (4). In several of the following experimental tests filter paper and a nutrient solution are required. Unless stated to the contrary, the filter paper used was Reeve Angel no. 202, circles 25 cm. in diameter. The following stock solutions were prepared and used in these tests.

NH ₄ -tartrate (40.912 gm. per liter).....	25 ml.
MgSO ₄ ·7H ₂ O (9.860 gm. per liter).....	5 ml.
KH ₂ PO ₄ (10.900 gm. per liter).....	5 ml.
Dextrose (66.700 gm. per liter).....	15 ml.
Ferric tartrate (0.1 saturated solution).....	3 drops
H ₂ O (distilled).....	50 ml.
Total	100 ml.

The data presented in tables 1-3 represent several replicas; enough to assure a biological significance of the results (that is, the factor F represents the mean of nine original values).

Experimentation

I. EFFECT OF VARYING QUANTITY OF FILTER PAPER USED

In this test the filter paper quantity, which is specified in each of the following solutions, was separately shredded, suspended in water, autoclaved for 20 minutes at 15 pounds' pressure, cooled to room temperature, and filtered. These solutions are:

C = control (distilled H ₂ O only)		
1 part = $\frac{1}{8}$ piece filter paper in	50 ml. H ₂ O	
2 parts = $\frac{1}{4}$ piece filter paper in	100 ml. H ₂ O	
4 parts = $\frac{1}{2}$ piece filter paper in	200 ml. H ₂ O	
8 parts = 1 piece filter paper in	400 ml. H ₂ O	
16 parts = 2 pieces filter paper in	800 ml. H ₂ O	
32 parts = 4 pieces filter paper in	1600 ml. H ₂ O	

Each of these aqueous solutions was filtered and reduced to 50 ml. by partial evaporation, prior to testing. This was then combined with the other stock solutions (with 25 ml. NH₄-tartrate solution, 5 ml. MgSO₄·7H₂O solution, etc.). This procedure yielded 100 ml. of each test solution. These were portioned into 25-ml. aliquots and placed in 125-ml. Erlenmeyer flasks, sterilized by autoclaving, inoculated with the *Rhizopus* spores, and cultured under the standardized conditions.

The results in table 1 confirm those obtained by NIELSEN and HARTELIUS (2, 3) and by DAGYS (1), suggesting that filter paper contains a co-factor for certain

microörganisms. Although the extent of growth was related to the quantity of filter paper used, the increases were less than those previously reported (3). With this confirmation of the beneficial effect of filter paper, an effort was made to investigate the specific agencies involved.

TABLE 1
EFFECT OF INCREASED CONCENTRATIONS OF CO-FACTOR IN
FILTER PAPER ON GROWTH OF RHIZ. SUINUS

CULTURE	AVERAGE DRY WEIGHT OF RHIZOPUS MYCELIUM (MG).	F \pm SE	SPORULATION
Control.....	81.6	1.00	++
1 part.....	83.2	1.02 \pm 0.009	+++
2 parts.....	89.6	1.10 \pm 0.015	+++
4 parts.....	95.0	1.16 \pm 0.009	+++
8 parts.....	96.7	1.18 \pm 0.012	+++
16 parts.....	102.8	1.26 \pm 0.025	+++
32 parts.....	104.3	1.32 \pm 0.011	+++

II. EFFECT OF FILTER PAPER WHEN TREATED VARIOUSLY

In this series of experiments each of the following reagents was autoclaved with 50 ml. of water and $\frac{1}{8}$ sheet of filter paper. The paper was removed by filtration, and the filtrate, after cooling, was brought to mark. To this filtrate was added the remaining stock solutions, as in test I. The substances constituting the solutions of the original autoclavings are:

- C. No filter paper (control).
- I. Filter paper+all stock solutions.
- II. Filter paper+water.
- III. Filter paper+ NH_4 -tartrate solution.
- IV. Filter paper+ MgSO_4 solution.
- V. Filter paper+ KH_2PO_4 solution.
- VI. Filter paper+dextrose solution.
- VII. Filter paper+ferric tartrate solution.
- VIII. Filter paper ashed in muffle furnace; ash added to nutrient solution, prepared by combination of stock solutions; mixture agitated for 30 minutes, then filtered and autoclaved.
- IX. Similar to VIII, except filter paper treated with 5 ml. of concentrated HCl prior to ashing.

The data of table 2 show the following order for the various test solutions, given in their respective degree of effectiveness on the stimulation of dry matter production: (1) Autoclaving the filter paper with the NH_4 -tartrate solution and then adding the other nutrients gave the most effective solution. This was fol-

lowed closely by a solution prepared by treating the nutrients with a HCl-ash of the filter paper. (2) Significantly lower in effectiveness was the solution which resulted by autoclaving the filter paper with the dextrose solution alone and then adding the other compounds. (3) Filter paper autoclaved in water, when supplemented with the other compounds, gave the next lower stimulative power. (4) The ash of filter paper, plus all the nutrients, ranks next. (5) Four substances when autoclaved separately with filter paper produce solutions which constitute the fifth class: MgSO_4 , KH_2PO_4 , all stock solutions, and ferric tartrate. (6) The control, which contained no filter paper or products thereof, was markedly lowest.

TABLE 2
METHODS IN PRODUCTION OF SYNTHETIC SUBSTANCE B

CULTURE	AVERAGE DRY WEIGHT OF RHIZO- PUS MYCELIUM (MG.)	F \pm SE	SPORULATION
Control.....	79.8	1.00	++
I.....	98.3	1.23 \pm 0.009	++++
II.....	104.2	1.31 \pm 0.008	++++
III.....	113.4	1.42 \pm 0.018	++++
IV.....	100.8	1.26 \pm 0.010	++++
V.....	100.8	1.25 \pm 0.008	++++
VI.....	107.1	1.34 \pm 0.017	++++
VII.....	97.6	1.22 \pm 0.008	++++
VIII.....	102.9	1.29 \pm 0.011	++++
IX.....	110.5	1.39 \pm 0.018	++++

Since autoclaving the filter paper with either NH_4 -tartrate or dextrose resulted in a greater stimulating value than when filter paper was autoclaved with water alone, it might be inferred that the co-factor enters into an organic salt combination chemically to form a growth factor for the *Rhizopus*. No explanation is offered to account for the lower value obtained when all the stock solutions were autoclaved with the filter paper. Since the ash, and especially the HCl ash, will substitute for the filter paper itself, it is most probable that this co-factor is inorganic in nature.

III. FURTHER METHODS IN PRODUCTION OF SYNTHETIC SUBSTANCE B

It was realized from the preceding that the reautoclaving, necessary for sterilization, introduced a factor which precluded definite proof that this co-factor did combine with one of the organic compounds and not with one or more of the compounds used. It was deemed advisable, therefore, to prepare and to test the following solutions:

- C. Control.
- I. Filter paper autoclaved with all stock solutions, then removed by filtration and filtrate sterilized and tested.
- II. Similar to I, except that the Reeve Angel filter paper was replaced by an "ash-free" paper, Whatman no. 42.
- III. Filter paper and NH_4 -tartrate solution autoclaved and paper then removed by filtration. Filtrate sterilized by autoclaving. Remaining stock solutions mixed in proportions specified and heat sterilized, then mixed and tested.
- IV. HCl-ash suspended in water for 30 minutes, filtered, and filtrate retained. Stock solutions added to filtrate and mixture heat sterilized. Resultant solution tested.
- V. HCl-ash suspended in water for 30 minutes, filtered, and filtrate sterilized by autoclaving. Stock solutions mixed and heat sterilized, then mixed and resulting mixture tested.

TABLE 3
METHODS IN PRODUCTION OF SYNTHETIC SUBSTANCE B

CULTURE	AVERAGE DRY WEIGHT OF RHIZOPUS MYCELIUM (MG.)	F \pm SE	SPORULATION
C.	84.1	1.00	++
I.	107.8	1.28 \pm 0.015	++
II.	98.6	1.17 \pm 0.013	+++
III.	86.9	1.03 \pm 0.019	++
IV.	102.6	1.22 \pm 0.022	+++
V.	73.4	0.87 \pm 0.015	+++

The data of table 3 indicate that the slight augmentation in yield for the culture growth on test solution III, as compared with the control, is without significance. This fact tends to prove that the autoclaving of filter paper with the NH_4 -tartrate solution does not result in a chemical formation of substance B. However, it does result in the growth factor formation when both the nitrogen- and carbon-nutrient sources are present, as shown by the results with the first two solutions. A tentative explanation for the discrepancy between the stimulative powers of the NH_4 -tartrate treated solutions in the two tests is as follows, for the value of 1.42 for culture III in table 2 and for the value of 1.03 for culture III in table 3. In the former test, the second autoclaving permitted a reaction, at high temperatures, between the soluble substances of the filter paper and all the compounds used. In the latter test, a reaction of the NH_4 -salt and the paper alone was possible at the higher temperatures. A comparison of these two results would lead to the inference that the autoclaving of NH_4 -tartrate and filter paper does not result in a significant production of synthetic substance B.

The acceleration of growth resulting from the use of the "ash-free" paper, Whatman no. 42, as compared with the other, indicates the possibility that perhaps the co-factor is not metallic. This ash-free paper does contain, however, traces of metallic ions and leaves a small but perceptible ash, when treated in a muffle furnace. A comparison of the results obtained with test solutions IV and V gives an interesting picture of the possible mechanism involved. The metallic ions, obtained by ashing filter paper, are toxic in themselves but do result in the formation of a marked stimulator when autoclaved with the stock solutions.

Tentative conclusions

1. Commercial filter paper contains a water-soluble co-factor for *Aspergillus niger* and *Rhizopus suinus*.
2. This so-called co-factor is possibly metallic in nature and tends to be toxic to the vegetative growth of *R. suinus*, when used singly.
3. The co-factor enters into the synthetic formation of a growth factor, of the substance B group, when autoclaved with an organic ammonium salt and a sugar.
4. This chemically formed substance B may be an organic salt of some trace element, which is in accord with the suspected nature of the biologically produced substance B.

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LITERATURE CITED

1. DAGYS, J., Wuchsstoffe der Mikroorganismen in embryonalen Geweben und in Blutungs-säfte. *Protoplasm* 24:14-91. 1935.
2. NIELSEN, N., and HARTELIUS, V., Ueber die Bildung eines Wuchsstoffes (Gruppe B) auf chemischem Wege. *Biochem. Zeitschr.* 256:2-10. 1932.
3. ———, Untersuchungen über die Wirkung einiger Metalle als Co-Wuchsstoffe. *Biochem. Zeitschr.* 259:340-350. 1933.
4. WORLEY, C. L., Agencies affecting production of substance B by *Rhizopus suinus*. *Plant Physiol.* 16:461-480. 1941.

EFFECT OF VITAMINS ON GROWTH OF RADISH AND CAULIFLOWER¹

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It has been stated that the use of vitamins B₁ and B₂ added in solution to intact plants grown in sand or gravel with added nutrients results in increased growth (1, 2). Others have not been able to obtain these effects (3, 4), either in sand culture or in ordinary garden soil. In most of these experiments the concentration of B₁ used was 0.01 mg. per liter of solution, with a few reporting quantities up to 1 mg. per liter. This paper reports the effects of higher concentrations.

Experiment with radish

Eight-inch clay pots were painted on the inside with a special nontoxic asphalt paint and filled with a no. 1, fine quartz sand. The hole in the bottom of each pot was plugged with glass wool. All pots were leached three times with distilled water previous to planting the seeds.

Approximately twenty untreated seeds of Scarlet Globe radish were planted in each pot to a uniform depth, and these were later thinned to an even stand of eight plants per pot. Each treatment consisted of five replications, so that there was a total of forty plants per treatment. The pots were randomized on the greenhouse bench by drawing numbers out of a box.

The experiment was set up in the 50°-60° F. greenhouse on November 28, 1940, at Ithaca, New York. The mean daily temperature was close to 57° F. No additional light was given, the plants receiving only that normal for the period. With the exception of a few sunny days the weather was mostly overcast during the growing period, there being 27 per cent of possible sunshine in December and 27 per cent in the following month up to January 22.

Hoagland's nutrient solution plus minor elements were added at intervals of 3 or 4 days in amounts sufficient to leach through the pot. Distilled water was added on a few occasions, usually after a sunny day. All pots were thoroughly leached with distilled water once a week.

Pure crystalline vitamins B₁, B₂, and B₆ were used. Fresh solutions were made up each time the pots were treated by weighing out the necessary number of milligrams of the vitamin and adding a sufficient amount of distilled water so that 1 cc. of the solution contained 1 mg. of the vitamin. From these stock solutions dilu-

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tions were made for those treatments requiring smaller amounts of the vitamins. Vita Flor, a commercial compound containing 0.1 per cent of vitamin B₁, 0.5 per cent nicotinic acid, and traces of vitamins B₂, B₆, and pantothenic acid, was also included.

The vitamins were added to sufficient water to make 300 cc. of solution per pot per treatment, while the checks were given 300 cc. of distilled water. Additions of the vitamins were generally made on the morning following application of the nutrient solution. Vitamins were applied on December 5, 9, 14, 17, 20, and 23, 1940, and January 1, 4, 8, 12, and 15, 1941. The radishes were harvested on January 22.

Growth of the plants was steady, considering the amount of cloudy weather prevalent during the short days of winter. A slight light green area was noticed around the margin of the leaves of many of the plants. This cannot be attributed to the treatment as it was present also on the adjacent greenhouse bench where the same seed was used in a fertilizer experiment in soil with different levels of N, P, and K. No one fertility level had more leaves with light green margins than another, so apparently this was a genetic factor rather than nutritional.

To check the effect of the vitamin applications on the pH of the sand, composite samples were taken several hours after treatment from those pots receiving the highest applications of vitamins. The determinations were made by the quinhydrone method, and the pH was found to average 5.45-5.90, with the checks at 5.73.

When the plants were harvested on January 22, the majority of the roots were of ordinary market size. The eight plants were pulled from each pot, the roots carefully shaken in water to remove adhering sand and then dried with paper toweling. Weights were made on a balance sensitive to 0.01 gm.

Table 1 shows the number of milligrams of vitamins applied per pot per treatment. The figures for the fresh and dry weights of the plants are the means for each treatment, consisting of five pots of eight plants each. While some individual treatments resulted in significant increases, none was significantly greater or less than the checks. No treatment, even those where 10 mg. of the vitamin was applied per pot per treatment, had a significantly depressing effect on growth. Apparently 10 mg. of vitamins per pot was entirely without effect, either beneficial or detrimental.

Experiment with cauliflower

Cauliflower seeds of the variety Super Snowball were planted on November 24, 1940, in flats of pure, washed, fine quartz sand. On December 11 they were transplanted to 5-inch new clay pots that had been previously painted on the inside with the nontoxic asphalt paint.

Ninety-six pots were placed in both the 60°-70° and 70°-80° F. greenhouses.

The ninety-six pots in each house were divided, one-half being put on long day from January 11, 1941, to harvest on February 6, 1941, by using four 100-watt bulbs suspended approximately 18 inches above the surface of the leaves from 5:00 to 11:00 P.M. Black cloth curtains hung on the four sides at 5:00 P.M. each day prevented the light from affecting adjacent plants.

Twelve pots at each temperature from both the long- and short-day treatments were reserved as checks. Twelve were treated with 0.01 mg. of vitamin B₁, twelve with 0.1 mg., and twelve with 0.5 mg. on the following dates: December 14, 19, 23, and 28, 1940, and January 3, 7, 11, 15, 20, and 25, 1941.

TABLE 1

TREATMENT	FRESH WEIGHT (GM.)			DRY WEIGHT (GM.)*	
	WHOLE PLANTS	ROOTS	TOPS	ROOTS	TOPS
0.01 B ₁	49.58	27.46	22.10	1.45	2.00
0.5 B ₁	48.42	27.90	20.52	1.54	1.81
1.0 B ₁	40.14	19.90	20.24	1.26	1.92
5.0 B ₁	49.48	27.04	22.24	1.48	1.98
10.0 B ₁	48.86	26.02	22.84	1.21	2.13
0.01 B ₂	47.50	25.64	21.86	1.44	1.85
0.5 B ₂	48.72	24.18	24.54	1.36	2.34
1.0 B ₂	50.26	25.66	24.60	1.40	2.13
5.0 B ₂	47.12	25.36	21.76	1.17	1.95
10.0 B ₂	38.66	18.16	20.50	1.11	1.93
0.01 B ₆	46.18	24.04	22.14	1.58	2.07
0.5 B ₆	45.34	24.80	20.36	1.41	1.98
1.0 B ₆	44.56	23.64	20.92	1.29	1.99
5.0 B ₆	44.56	22.06	22.50	1.28	2.18
10.0 B ₆	42.38	22.08	20.30	1.40	1.77
Vita Flor 1 cc.....	40.22	19.98	20.24	1.17	1.97
Vita Flor 2 cc.....	39.52	21.10	18.42	1.18	1.96
Vita Flor 5 cc.....	36.02	17.26	18.76	0.98	1.98
0.01 B ₁ , B ₂ , and B ₆	44.22	23.58	20.64	1.27	1.96
0.1 B ₁ , B ₂ , and B ₆	47.90	27.58	20.32	1.50	1.99
Check.....	43.60	25.68	17.92	1.36	1.70

* Differences not significant. Difference of 11.93 necessary for significance at odds of 19:1.

Hoagland's complete nutrient solution plus minor elements were added to the pots approximately every fourth day, and when necessary distilled water was added between the nutrient applications. Freshly made vitamin solutions were used, dilutions being made from a stock solution containing 1 mg. of the pure crystalline vitamin per cubic centimeter of solution. When the vitamins were applied, they were so diluted that each plant received 10 cc. of distilled water with the vitamin included. It was felt, especially during the early stages of growth when the plants were small, that if any effect was to be obtained, 10 cc. of the solution would be more effective than the same amount of vitamin in a larger amount of water.

Growth was steady in both houses, and a few days after the lights were used increased growth was evident on those plants receiving the extra light.

All plants were harvested on February 6. Weights were taken on a balance sensitive to 0.01 gm. and the data subjected to analysis of variance. In neither house was there any significant difference between the treatments and their checks.

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LITERATURE CITED

1. BONNER, J., and GREENE, J., Vitamin B₁ and the growth of green plants. BOT. GAZ. 100: 226-227. 1938.
2. DENNISON, R., Growth response of plants to Riboflavin and ascorbic acid. Science 92:17. 1940.
3. HAMNER, C., Effect of B₁ on the development of some flowering plants. BOT. GAZ. 102:156-168. 1940.
4. MINNUM, E. C., Effect of vitamin B₁ on the yield of several vegetable crop plants. Amer. Soc. Hort. Sci. Proc. 38:475-476. 1941.

NUMBER OF CHLOROPHYLL COMPONENTS

F. P. ZSCHEILE

In 1934 the writer presented certain evidence (8) which indicated that normal green leaves might contain a third component, *c*, in the chlorophyll complex, in addition to components *a* and *b* which were originally found by WILLSTÄTTER and STOLL (6). The evidence for this additional component was incomplete and inconclusive. BAKKER (1), WINTERSTEIN and SCHÖN (7), and MACKINNEY (4, 5) presented evidence unfavorable to ZSCHEILE's suggestion of a third component. In the meantime, after the development of more refined methods for chlorophyll preparation and the availability of better absorption data on the products formed by treatment of chlorophyll with acid, the writer has re-investigated this problem and considers that a note is desirable to clarify the subject.

In 1936, after the criticisms of WINTERSTEIN and SCHÖN (7) concerning the use of talc were at hand, components *a* and *b*¹ were prepared with sucrose substituted for talc, other conditions remaining much the same as described in 1934 (8). All solutions were kept at 5° C. except while being studied. Sucrose was washed from the ethereal chlorophyll eluates with water. To force the solution through the adsorbent column, a pressure of 5 lb. of nitrogen was used instead of suction. The use of glass columns approximately 10 inches long permitted their more exhaustive washing.

The zones formed were very similar in color to those reported by ZSCHEILE with talc. Some brown pigment separated out at the top of the column and was discarded. The characteristic "*c*" fraction, green in color, was isolated by adsorption on a second sucrose column (5 inches in length). It separated completely from the 5-mm. zone of brown pigment, which remained at the top of this column. No blue layer of *a* appeared at the bottom. The *a* fraction, after adsorption on a second column, produced no brown layer whatever and was uniformly blue-green on the column, yielding a blue ether solution. The *b* fraction from extracts 11-18 (8) was dissolved in 30 per cent ether—70 per cent petroleum ether and chromatographed upon a sucrose column. No blue layer of *a* appeared until the column was developed with 40 per cent ether plus 60 per cent petroleum ether. Then a narrow 5-mm. band of blue *a* separated completely from the green at the bottom of the column. The lower 15 mm. of the green zone was lighter in color than the main body (140 mm.) and was discarded because of possible contamination with the *c* fraction. A thin brownish region at the top was also discarded. All samples were

¹ These preparations were made in the department of chemistry, University of Chicago.

washed four times with water while dissolved in ether. Most of the solvent was then evaporated under reduced pressure, and the sample was dried *in vacuo* at a pressure of 2×10^{-5} mm. of mercury, as measured on a Macleod gauge.

Absorption measurements were then made on ether solutions with the photo-electric spectrophotometric apparatus described by HOGNESS, ZSCHEILE, and SIDWELL (3). A mixture of ether solutions of these component preparations (*a* and *b*) containing 48 per cent *b* by weight was studied. Its visible absorption curve was intermediate between the curves for the single components, and the curve for the mixture agreed closely with the others at their crossing points, indicating that the components of a mixture may be identified from its absorption curve if such curves for the components are known. The *a* fraction was washed four additional times with water, to remove any possibility of sucrose contamination, and dried. Absorption values at the principal maxima were lowered 4 per cent by this treatment.

A few drops of 22 per cent HCl were added to ether solutions of *a* and *b* to cause pheophytin formation. Curves of these products of acid treatment resembled those recently published by ZSCHEILE and COMAR (9).

The *c* fraction, which was apparently separated completely from *a* and from brown decomposition products on the sucrose column, was next considered. Its absorption curve was similar to those of earlier preparations from talc columns, so far as positions of maxima were concerned. It was noted that the maxima in the blue and red regions corresponded in wave length exactly to those of both chlorophyll *b* and "pheophytin *a*." Moreover, when the absorption curve for the *c* fraction was raised by multiplication of the absorption coefficients by the necessary factor 1.27 (presumably due to colorless impurities) to make it cross the other curves at one of their crossing points, 4210Å (4235Å according to 9), it was found that all three curves agreed closely at all the crossing points for chlorophyll *b* and pheophytin *a*. There were five such points in the visible spectrum. The shape of the curve for the *c* fraction indicated that this fraction consisted chiefly of chlorophyll *b* and pheophytin *a*, with the latter predominating. All the principal bands for these two compounds were present as maxima, and those for pheophytin *a* were more intense than those for chlorophyll *b*. Other combinations of chlorophyll and "pheophytin" pigments are eliminated by these considerations. The absorption spectra thus demonstrated that a homogeneous zone on an adsorption column may, under certain conditions, contain two adsorbed compounds.

Recent work in these laboratories has indicated several sources of error in the results of 1934 (8). Some of these were recently discussed by ZSCHEILE and COMAR (9), whose experiments have indicated the probable reasons for ZSCHEILE's 1934 results. The fact that all chlorophyll preparations from the writer's laboratory were dried previous to 1939 makes further detailed comparison of results futile.

It is concluded that the earlier observations on component *c* were the result of the decomposition of chlorophyll *a* to pheophytin *a* and of the fact that the chromatographic methods employed were inadequate to separate them. After consideration of more recent results (9, 2) it would seem that both the use of talc and the long time of preparation required in the earlier method were factors which could have caused the formation of pheophytin *a*. The advantage and usefulness of the spectrophotometric method were demonstrated as means of control during chromatographic separations.

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LITERATURE CITED

1. BAKKER, H. A., Purification of chlorophyll. Proc. Kon. Akad. Wetensch. Amsterdam 37: 679-684. 1934.
2. COMAR, C. L., and ZSCHEILE, F. P., Analysis of plant extracts for chlorophylls *a* and *b* by a photoelectric spectrophotometric method. Plant Physiol. 1942.
3. HOGNESS, T. R., ZSCHEILE, F. P., JR., and SIDWELL, A. E., JR., Photoelectric spectrophotometry: An apparatus for the ultra-violet and visible spectral regions; its construction, calibration, and application to chemical problems. Jour. Phys. Chem. 41:379-415. 1937.
4. MACKINNEY, G., Some absorption spectra of leaf extracts. Plant Physiol. 13:123-140. 1938.
5. ———, Criteria for purity of chlorophyll preparations. Jour. Biol. Chem. 132:91-109. 1940.
6. WILLSTÄTTER, R., and STOLL, A., Investigations on chlorophyll. English transl. by SCHERTZ, F. M., and MERZ, A. R., from the German. Science Press Printing Co. 1928.
7. WINTERSTEIN, A., and SCHÖN, K., Fraktionierung und Reindarstellung organischer Substanzen nach dem Prinzip der chromatographischen Adsorptionsanalyse. III. Mitteilung: Gibt es ein Chlorophyll C? Zeitschr. physiol. Chem. 230:139-145. 1934.
8. ZSCHEILE, F. P., JR., An improved method for the purification of chlorophylls *a* and *b*; quantitative measurement of their absorption spectra; evidence for the existence of a third component of chlorophyll. BOT. GAZ. 95:529-562. 1934.
9. ZSCHEILE, F. P., and COMAR, C. L., Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. BOT. GAZ. 102:463-481. 1941.

CURRENT LITERATURE

A Revision of Melanconis, Pseudovalsa, Prosthecium, and Titania. By LEWIS E. WEHMEYER. Ann Arbor: University of Michigan Press, 1941. Pp. 161. Pls. 11. \$2.50.

This is an important contribution to our knowledge of a difficult group of the Pyrenomycetes. The introduction sketches the history of the genera treated and discusses the criteria used in segregating the different groups. The author realizes that there are in most cases no fixed lines of demarkation between genera and species. The current system of classification, based chiefly on spore color and septation, is artificial and leads to confusion of natural groups. Natural relationships can be determined only by study of the complete life cycles and microscopic characters of the plants. The author concludes that genera "are almost impossible to define," and "A genus, therefore, is generally an artificial conception, has arbitrary boundaries and should be used in a manner to serve most efficiently the two purposes mentioned—as an indication of relationships and as a convenient index tab."

An attempt to attain this laudable purpose could hardly be expected to result in a perfect agreement with other students of the group. The author's generic concepts are commendably conservative. New genera and species, frequently numerous in such revisions, are noticeably lacking; such new names as are used are mostly applied to subgeneric and sectional groups. The adoption of *Prosthecium* for species found heretofore mostly in *Aglaospora* and *Pseudovalsa* results in a number of new combinations. The chief difference between *Prosthecium* and *Pseudovalsa* is the presence of a hyaline appendage on each end of the ascospores. Whether this character is of generic value is a matter of opinion. The demonstration of such appendages depends in many cases upon the age and condition of the specimen. The separation of *Massaria* from *Aglaospora* on the 4-spored asci of the latter genus is properly recognized as doubtful. Cultural and cytological studies are needed to clear up this point.

The author has had unusual opportunity to study type and authentic specimens of most of the older species in this group of fungi and is thus able to give very complete synonymy. It is unfortunate that the specimens in the mycological collections of the Bureau of Plant Industry were not examined, as they would have given additional information regarding the distribution of the species in this country and perhaps the identity of some of the still doubtful ones. Rather too serious attention seems to have been given to the frequently modified and still unstable rules of nomenclature. This has led to the rejection of some of our old and generally used specific names. For example, the common species *Aglaospora profusa* (Fr.), published on p. 392, Fr. Syst. Myc. 2, has been replaced by *A. anomia* (Fr.), published on p. 381 of the same work. It is to be hoped that some day, in the interests of uniformity and stability, mycologists will abandon this procedure in regard to specific names, as they are already doing with generic names, and conserve the well-known ones. Such changes lead only to confusion and are a detriment to the advancement of systematic mycology, and it would seem preferable to delay making them for the present, as some of our British colleagues are doing, hoping that the next Botanical Congress will approve the conservation of specific names.

This excellent monograph adds extensively to our knowledge of the older species and their synonymy and also of their conidial stages, which have in many cases been proved by pure

cultures. It is a contribution of such importance and value that criticisms of it seem trivial.—C. L. SHEAR.

Die Methoden der Fermentforschung. Edited by EUGENE BAMANN and KARL MYRBÄCK. Leipzig: Georg Thieme, 1940-1941.

This compendium of methods of enzyme research began to appear early in 1940, and to date eight Lieferungen have appeared, totaling 3047 pages. Each section of the monograph has been prepared by some worker familiar with the special methods employed in some restricted field of enzyme chemistry. The introductory section considers the nature of enzymes, the mechanism of enzyme reactions, a general discussion of methods of enzyme research, and the nomenclature and systematic classification of enzymes.

The following special sections are three in number. The first deals with the substrates used in enzyme research, their preparation, properties, and applicable investigational techniques; the intermediate products formed during reactions; and the end products. This section also discusses the more recent research methods used in substrate investigations and the general methods of research on enzymes, such as methods of following catalysis, obtaining and testing enzyme preparations, isolation and characteristics of lyo- and desmo-enzymes, general procedures in concentration and separation of enzymes, and the determination of the general properties of enzymes.

The second division takes up special groups of enzymes, such as the hydrolases, the desmolases, and enzymes of biological oxidation and reduction, assimilation, antienzymes, and enzyme models.

The third section is devoted to technical research on enzymes in industry, such as fermentation of grains, etc., processing of fats, manufacture of malt extracts, and the baking industries. The last part of Lieferung 8 considers research methods used in clinical problems.

This excellent compilation should be in the hands of, or accessible to, all students of enzyme reactions. Naturally it is impossible for such a compendium to attain completeness or perfection, but a work which draws together thousands of papers which have appeared in periodic literature has exceptional value. About 130 individual scientists have co-operated in this effort, and approximately 200 separate reports are included.

It is remarkable that in a world so torn with strife, scientific men still go forward serving the arts of peace. This fine example of co-operation is worthy of the highest commendation. The total price of the eight Lieferungen issued is RM 233.7, or somewhat less than \$100. Because of the great expense of publication, the printer cannot offer them except in complete sets.—C. A. SHULL.

The Cytoplasm of the Plant Cell. By ALEXANDRE GUILLIERMOND. Translated by LENETTE ROGERS ATKINSON. Waltham, Mass.: Chronica Botanica Co.; New York City: G. E. Stechert and Co., 1941. Pp. 247. \$4.75.

This volume, which deals with the cytoplasm and its inclusions, is characterized by the thoroughness with which it has been done, the clarity of presentation, and the fairness with which the older and newer views in cytology are brought together and integrated.

There is a succinct and informative foreword by SEIFRIZ, following which there are twenty chapters. The first presents a historical sketch of the general subject, points out the difficulties in the study of cytoplasm, and recommends methods. The second is devoted to general facts on the structure of the plant cell, its cytoplasm and morphological constituents, while the follow-

ing three chapters give detailed attention to the physical properties and general characteristics, chemical constituents, and physico-chemical constitution of the cytoplasm. Six chapters are next concerned with plastids and the chondriome, the relationship between chondriosomes and plastids, the duality of the chondriome, and hypotheses relative to the roles of chondriosomes and plastids. Vacuoles, including methods of vital staining, development, origin, and significance, and the role of the vacuolar system and hypotheses concerning it, are discussed in the succeeding five chapters. Golgi apparatus, canaliculi of Holmgren and other cytoplasmic formations, lipside granules, microsomes and other metabolic products, and cytoplasmic alterations occupy the next three chapters. A general summary concerning the present knowledge of the cytoplasm, the chondriome, plastids, and the vascular system or vacuome is given in the final chapter.

The present volume is the first addition, printed in America, to the list of books which Dr. Frans Verdoorn is editing and publishing under the title, *A New Series of Plant Science Books*. MRS. ATKINSON has accomplished an excellent job of translating and interpreting for English readers a highly stimulating work, which can be recommended to botanists generally.—J. M. BEAL.

Native Midwestern Pastures; Their Origin, Composition and Degeneration. By J. E. WEAVER and W. W. HANSEN. University of Nebraska Conservation and Survey Division: Nebraska Conservation Bulletin no. 22, 1941.

Regeneration of Native Midwestern Pastures under Protection. By J. E. WEAVER and W. W. HANSEN. University of Nebraska Conservation and Survey Division: Nebraska Conservation Bulletin no. 23, 1941.

These two papers represent another step forward in the careful studies by which WEAVER and his students have made the central portion of fragmented true prairie the best known of all native grasslands. In the first, five stages in the degeneration of native prairie under the influence of grazing by domestic stock are set forth, together with the various types of vegetation resulting. This task, based originally on studies begun in 1929, was made more difficult by the effects of the droughts of 1934-40 in accelerating degeneration or in causing the almost immediate destruction of some types. The earlier survey permitted an evaluation of the responses of the various species to grazing alone; and the conclusions, confirmed by clipping experiments, are embodied in lists and discussions of prairie grasses and forbs that decrease under grazing, those that increase, and grasses and forbs that invade pastures. The changes in pasture types that have resulted from drought are clearly set forth. The second bulletin deals with the changes in the plant population over a 4-year period, 1937-40, which resulted from the complete protection from grazing of a pasture near Lincoln, classed in 1932-33 in the third stage of degeneration. The recovery of the prairie grasses, based on stem counts in permanent quadrats, is excellently depicted in graphs.

This study also includes a comparison of the yields, in 1940, of pasture under protection for 1, 2, and 4 years, and prairie. Based on clipping at various intervals, yields were 1.15, 1.51, 1.31, and 1.12 tons per acre, respectively. When evaluated on a probable utilization basis, the comparative figures are 0.49, 0.72, 0.74, and 0.76 tons. Based on the better forage grasses, one year of complete protection more than doubled the production of usable material, and 3 years of protection increased it eight times.—C. E. OLMSTED.

- ✓ *Genetics and the Origin of Species*. 2d ed. revised. By THEODOSIUS DOBZHANSKY. New York: Columbia University Press, 1941. Pp. xviii+446. \$4.25.

Although only 4 years have elapsed since the first edition of this highly stimulating book appeared, such progress has occurred in certain areas, notably in population genetics and cytogenetics, that not only substantial additions but complete rewriting of certain portions of the book were found necessary. The organization and the chapter headings of the former edition have been retained, and the book has been extended by some 82 pages.

Again the author has succeeded in bringing into proper relation to one another the accumulation of information on mutations of all sorts (genic and chromosomal), on genetic composition of populations, on species differences, and on the mechanism of hybrid sterility.

The volume comprises an extraordinary amount of factual material, but this is so thoroughly organized and integrated as to make the book highly readable throughout. As in the former edition, one of the most valuable features is the incorporation of the work of European—and especially of Russian—authors, whose results have often been overlooked in America. It is a necessary book for all who are interested in recent developments of the theory of evolution.—J. M. BEAL.

- Phylogenetic and Cytological Studies in the Ranunculaceae*. By WALTON C. GREGORY. Trans. Amer. Phil. Soc., New Series, Vol. XXXI, Part V. 1941.

A study of the phylogeny and systematics of the Ranunculaceae, chiefly on the basis of the type and number of chromosomes. In all, the chromosomes of 109 species involving 19 genera have been studied, with 43 species from 8 genera being reported for the first time. The following basic chromosome numbers have been selected for the family: 5, 7, 8, 9, and 13. The highest degree of polyploidy has been reached in the 7- and 8-series, with the highest $2n$ numbers 154 and 128, respectively.

An evaluation of the significance of polyploidy and of the relative roles of polyploidy as against other factors in evolution of the woody habit in the Ranunculaceae has been attempted. A phyletic rearrangement of the genera and tribes of the family has been made on the basis of chromosome type, size, and basic number. On the same data a polyphyletic origin of the various tribes of the family from some ancestral group has been suggested and illustrated by phylogenetic charts, comparing the classical and the author's classifications.—J. M. BEAL.

- ✓ *Cytology, Genetics, and Evolution*. By M. DEMEREC *et al.* Philadelphia: University of Pennsylvania Press, 1941. Pp. v+168. \$2.00.

Contains twelve addresses presented at the University of Pennsylvania's Bicentennial Conference, September, 1940: The nature of the gene, by M. DEMEREC; Chromosome structure, by CHARLES W. METZ; The sex chromosomes: heteropycnosis and its bearing on some general questions of chromosome behavior; chromosomal interchanges, by ALBERT F. BLAKESLEE; Chromosomal differences between races and species of *Drosophila*, by TH. DOBZHANSKY; Evolution of the germplasm, by CLARENCE E. MCCLUNG; Hereditary status of the rhizopods, by HERBERT S. JENNINGS; Nuclear behavior and reproduction in ciliated protozoa, by WILLIAM F. DILLER; Inheritance in ciliated protozoa, by T. M. SONNEBORN; The physico-chemical properties of the nucleus, by LEON CHURNEY; The chromosomes of the amphibian nucleus, by WILLIAM R. DURVEE; Radiation and the cell nucleus, by PAUL S. HENSHAW.—J. M. BEAL.

- ✓ *Tabular Keys for the Identification of the Woody Plants.* Compiled and arranged by FLORENCE B. ROBINSON. Champaign, Ill.: Garrard Press, 1941. Pp. iv+156. Spirally-bound, loose-leaf, planographed. \$2.50.

These keys are intended for field use and for quick reference to 500 trees, shrubs, vines, conifers, and broad-leaved evergreens growing in the northern states and Canada. There are separate groups of dichotomous keys to the genera for each of the groups of plants just named and for the leafy and the winter condition separately. The species are determined by referring to tabular keys of each genus, alphabetically arranged. Instruction in the use of these keys, a glossary, and nomenclatural lists of species, together with the common names, add to the usefulness of this work.—P. D. VOTH.

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NITRATE AND CARBOHYDRATE RESERVES IN RELATION TO NITROGEN NUTRITION OF PINEAPPLE

G. T. NIGHTINGALE

(WITH FOURTEEN FIGURES)

Introduction

Chemical analyses of leaves and other organs of horticultural crops for nitrogen, phosphorus, and potassium have shown that often—with critical selection of samples to be analyzed—there may be obtained reasonably accurate indices of the concentration of these nutrients in the plant. As applied to agriculture, this has often led to increased efficiency in the use of fertilizers (3, 4, 11, 27, 32). If employed as a practical guide to fertilizer requirements, however, such analyses involve the assumption that leaf values for N, P, and K that were correlated with best yields in any year or series of years should be considered as standards in successive years, at least in the same location.

Perhaps when water is not a limiting factor, and if there are locations where temperature and sunshine do not vary greatly from year to year, such an assumption may be adequate for practical purposes. In Hawaii, however, even in the same section of any given plantation, the amount of nitrogenous fertilizer required for maximum yields of pineapples has been found to vary by as much as 75 per cent in successive years. The nitrate reserve which must be present in the leaves of the plants for best yields varies in different years and in the same year in different locations. Indirectly, the reason for this lies in the fact that the plants are grown under conditions of temperature that differ materially, at elevations from only a little above sea level to over 2000 feet. Light intensity varies from less than 2000 to about 8000 foot candles in different seasons and locations. In spite of these differences, and sometimes radical variations in rainfall, it has been possible to obtain quantitative records that, regardless of location and weather, furnish the

information necessary for precise, efficient use of fertilizer. That these plant records are not limited in their use to a single location or season is owing primarily to the fact that carbon dioxide is regarded as a nutrient, and that quantitative records of starch reserves are employed as an index of carbon dioxide availability. All nutrients are essential, of course, but, with the exception of water, carbon dioxide is by far the most abundant nutrient the plant receives.

The uncertainty of the degree of availability of carbon dioxide makes records of carbohydrate reserves in the growing plant particularly pertinent. It is just as essential to have a measure of stored starch as it is to have it of nitrate, phosphorus, and potassium. In fact, starch reserves determine the percentage of nitrate that may be advantageously carried in the pineapple plant. In turn, for greatest efficiency, phosphate levels in the plant should be adjusted on the basis of nitrate concentration, and similarly nitrate is not freely absorbed if potassium is low, etc. Thus there is a chain of interdependent relationships directly or indirectly associated with opportunity for carbon dioxide assimilation that necessarily varies with prevailing weather conditions.

Although for best economic results the importance of avoiding an imbalanced relationship between nitrate, phosphate, potassium, and other ions in the plant cannot be overemphasized, the present report is concerned largely with carbohydrate and nitrate reserves. The principles involved have been discussed in part by KRAUS and KRAYBILL (10), and methods of applying these principles to large-scale pineapple production over considerable acreages were developed, first in sand or water cultures and in some cases under controlled environmental conditions, with tomato, fruit trees, and other horticultural crops (16, 17, 18, 19). These principles, applied to pineapple, have shown that the concentration of nitrate which the plant is permitted to accumulate should be adjusted in relation to its starch reserves. A deficiency of either plant constituent is to be avoided. Standards for judging the amounts present in relation to any given functional level of the plant are to be described.

Together with climatological data, various records of plants are also taken, such as starch and nitrate determinations. The data indicate why a particular field or area may include plants deficient in starch, nitrate, or other nutrients. If plants are deficient in any essential constituent, it becomes important to know why. It is not enough simply to know that the condition exists. For example, if nitrate reserves are low because of suberized root tips and root hairs and consequent limited absorptive capacity, the practical indications are in contrast to that of a field with similar nitrate reserves in the plants but with root systems capable of more active absorption. Thus the purpose of quantitative records of the growing plants is primarily to measure limiting factors, not alone those that can be controlled or in-

fluenced, but also those that cannot be controlled. Such records permit purposeful, well-timed applications of fertilizer. They also help to prevent futile attempts to attain impossible objectives.

Observations

QUANTITATIVE FIELD RECORDS

In this initial paper—dealing with the field aspects of pineapple nutrition—that may be referred to in later reports, it is proposed to give a rather inclusive description of methods of obtaining records of plants in the field. A mature pineapple plant of the Smooth Cayenne variety is shown in some detail in figure 1. It is propagated vegetatively and is almost the only variety of commercial importance in Hawaii. Figures 2-5 show plants of various ages as they appear in the field: planted through mulch paper, in 2-row beds 100 yards in length, the beds terminating at a field road at each end.

Representative samples of the distal half of the stem of the slips when employed for planting are analyzed for percentage dry matter (a good index of carbohydrate reserves and capacity for root production), for phosphorus, potassium, and nitrate. These constituents may be stored in quantity in the slips, although the amount and proportions may vary greatly with different lots of planting material. As soon after planting as roots have become established and some new top growth made, quantitative records are taken at about monthly intervals until blossom buds appear about a year later. The time from planting to the appearance of blossom buds may vary greatly, depending upon many factors.

PLANT WEIGHT.—In a productive commercial pineapple field, plants vary relatively little as compared with many horticultural crops. Nevertheless, in any part of a field under consideration, three plants are selected. These are judged, for the particular date concerned, to be large, medium, or small in comparison with the remainder of the plants in the area. These plants are pulled up and weighed.

NUMBER OF LEAVES.—The number of leaves over 12 inches long on each of these three plants is recorded.

LENGTH \times WIDTH OF LEAF.—A leaf is selected for measurement on each of the three plants. Figure 6 illustrates on the left an immature leaf; on the right, one that is approaching senescence. Between these is a leaf, representative of those selected for measurement, that is almost fully expanded, except for the lateral points at the base. The width is determined at a point one-third the distance up from the base of this leaf. Because a leaf persists for many months, leaf dimensions tell little of the quality of the plant, but they do serve as an index of the growing conditions at the time the leaf concerned was expanding.

SELECTION OF PLANTS.—In several key locations in a field, the following records are taken from a pair of representative plant beds. Proceeding along the cultivated



FIG. 1.—Plant at about 21 months; leaves cut off to show axillary shoots which produce second crop, the first ratoon. (Axillary shoots may be employed for planting, together with so-called slips.) Small slips just visible along peduncle. Small knoblike rudimentary fruit is discarded from slip before planting.

space, five paces in from the road, a plant from the nearest row in the left-hand bed is recorded as being large, medium, or small, as the case may be. Ten paces farther along, a plant from the nearest row in the right-hand bed is selected, and so on, until the other end of the bed has been reached and ten plants have been



FIG. 2.—Pineapple plants at about 6 months, showing style of planting, through edge of black mulch paper, in 2-row beds with cultivator space between.

recorded, five from either bed. By analysis of variance there is thus obtained a reliable index of plant weight, number of leaves, and length \times width of the leaf described (fig. 6), for the beds concerned.

ROOT ANCHORAGE.—When the three plants are being pulled up, a spring-balance is employed to record the number of pounds required to uproot them. On the basis of these tests and experience over a considerable period of time, a moderate tug at each of the ten plants enables an experienced worker to record for each the proximate root anchorage in pounds. This gives an index of the extent of the root

system. Anchorage of 100 pounds is considered 100 per cent, although it may reach as much as 150 pounds.

WHITE ROOT TIPS.—These are indicative of the ability of the plant to absorb water and other nutrients. In several locations, small trenches are dug inside and outside the beds so that the character of the root system may be determined.

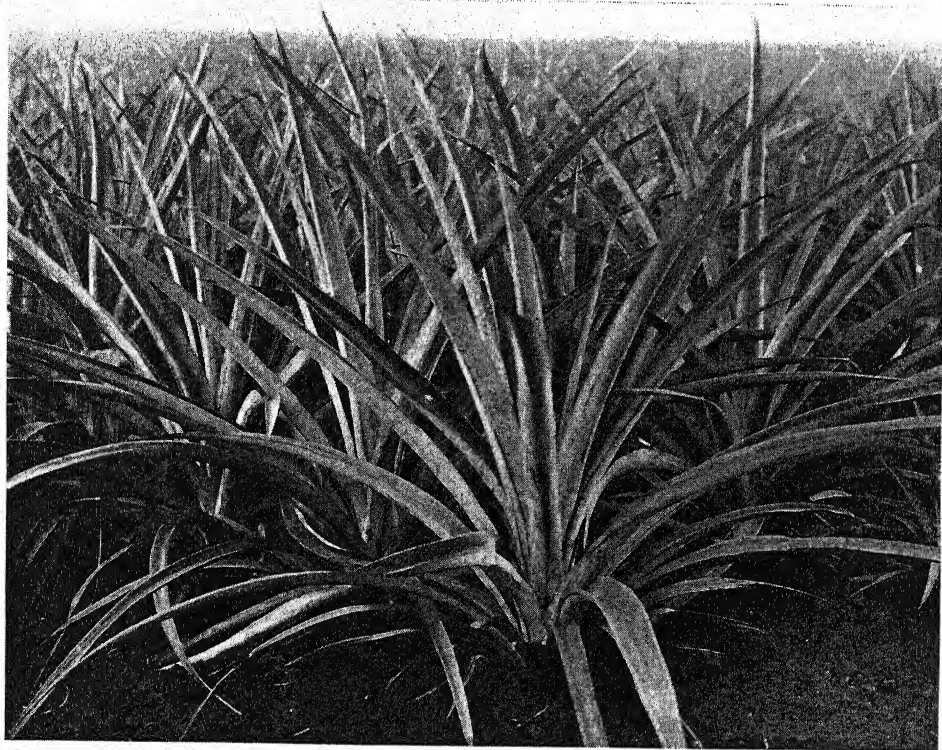


FIG. 3.—Pineapple plants at about 9 months. Along the troughlike upper surface of leaves, water from light showers or dew may flow toward center of plant, down through planting hole in mulch paper, to soil below. Fertilizer placed in lower axils of leaves is thus carried down and made available.

Samples for soil moisture are also taken at this time, usually under the edge of the mulch paper midway between the plants in the row at a depth of 6–8 inches. If all main roots and lateral roots observed have white root tips $\frac{1}{2}$ and $\frac{1}{4}$ inch or more in length, respectively, white root tips are recorded as 100 per cent. Anything less than this is indicated correspondingly on a percentage basis.

SEMI-MATURE ROOT TIPS.—Such root tips, recorded on a percentage basis, are slightly brownish, suberized, and relatively woody up to and often including the apex. They are incapable of adequate absorption of water, at least when evapora-

tion rates are high. Often within a day or two after soil conditions have become favorable, cell division may commence and new white root tips emerge apically. (Factors which singly or in combination may adversely influence the maintenance of active white root tips may be only mentioned here. They include lack of water, as when soil moisture is at or below the wilting percentage, probably high soil temperature [16, 30], and high soil solute concentration as shown by HAYWARD [6, 7] and others [19].)



FIG. 4.—Pineapple plant in blossom at about 15 months after planting

SENESCENT ROOT TIPS.—As implied, such root tips are dead or nearly so. If 100 per cent are in this condition, it means that it has been some time, probably weeks, since the plant has absorbed water and nutrients freely in adequate quantity—regardless of soil moisture content. It also means that, if the distal tissues of the roots are senescent, when soil conditions become favorable new white root tips will emerge relatively slowly, laterally well back on the main roots or as new adventitious roots from the base of the stem.

ROOT FACTOR.—To co-ordinate root condition in a single quantitative expression, the root factor is considered to be root anchorage in pounds \times percentage of white root tips. These records of root condition, while only proximately quantita-

tive, play a part in agricultural practice and in the interpretation of responses of pineapple plants in fertilizer experiments that is at least equal in importance to the more precise chemical analyses and other determinations to be described.

LEAF COLOR AND CARBOHYDRATES.—Often in a pineapple plant in which all the potential starch-storing cells are filled to capacity, macroanalyses—expressed as percentage either of fresh or of dry material—indicate relatively little starch.



FIG. 5.—Field nearly ready to harvest at about 21 months after planting

Hundreds of such macroanalyses of stems and leaves have been made, yet there has been no consistent correlation with starch deposits in the leaves that were easily seen by microscopic examination. The tests completely failed to indicate to what percentage of capacity the plant was supplied with starch. The reason is obvious. The pineapple plant frequently contains a very high proportion of inert mechanical fiber in both stem and leaves, and analysis of expressed juice, although of interest, does not include starch, the major carbohydrate reserve. Another plant type, relatively soft and succulent and with limited development of fibers, was—by microscopic observation—very low in starch. Yet as percentage of fresh or dry weight relatively more starch was indicated in such a plant than in the case of one

filled to capacity with this material. Of course, the absolute amount of starch could be easily computed from the conventional macroanalyses; but in nutritional studies, concentration is the index of degree of deficiency of carbohydrates, or any other material. Absolute amounts are correlated with size and not with quality of plant.

On the basis of microscopic tests, it soon became apparent that there was a consistent correlation between degree of greenness and starch reserves in the potential starch-storing cells of leaves. It should be recognized, however, that in a woody, highly differentiated leaf of relatively xeromorphic structure, the number of cells capable of storing starch are comparatively few and their protoplasmic volume usually relatively small. Nevertheless, if all these cells are approximately filled to capacity with starch, as in a yellow-green leaf, the carbohydrate rating is considered 100 per cent for that leaf. As already pointed out, this is in contrast to any figure that could be obtained by macroanalysis, where—on a percentage of fresh or dry-weight basis or as absolute amount in grams—the starch content would be recorded as very low for that leaf. It is recognized that this method of estimating carbohydrate reserves is a departure from the more conventional methods of expression employed by plant physiologists, but in principle it corresponds to expression on the basis of "protoplasmic mass" long used by animal chemists.

In any event, this method—together with analyses for nitrate—is useful in practice and gives a high degree of precision.

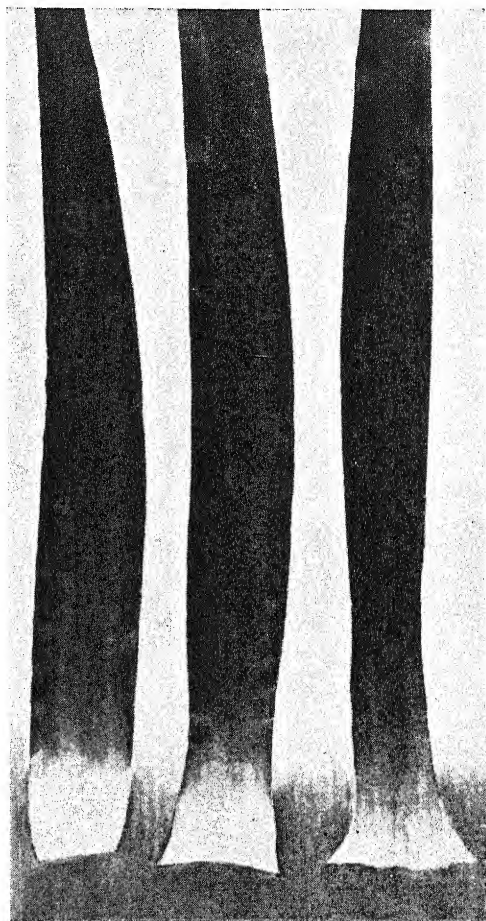


FIG. 6.—Left: immature leaf with relatively much white semi-meristematic tissue and comparatively narrow sides tapering inward slightly at base. Right: mature leaf with little white semi-meristematic basal tissue; base broad in relation to remainder of leaf, with pronounced lateral points forming acute angles. Center: leaf nearly fully expanded except at base; middle third of white basal tissue of leaves of this type employed for chemical analysis.

COLOR.—The color standards shown in figure 7 are recognizably distinct, but of course they intergrade insensibly. They represent an arbitrary median region of a color range for only the upper surface of leaves. Of this area, certain portions are excluded in making estimates of the leaf color of a plant for starch reserves. The areas omitted include the immature leaves, less than 9 inches long, in the center of the plant, the immature, relatively unexposed proximal third of longer leaves, and the distal third or tip of longer leaves. This terminal tissue is always relatively senescent after the very early stages of growth, and in any event is small in area because the leaf tapers to a point. Thus it is the color-starch relationship of only the potential starch-storing cells of approximately the middle third of the leaf that is referred to here.

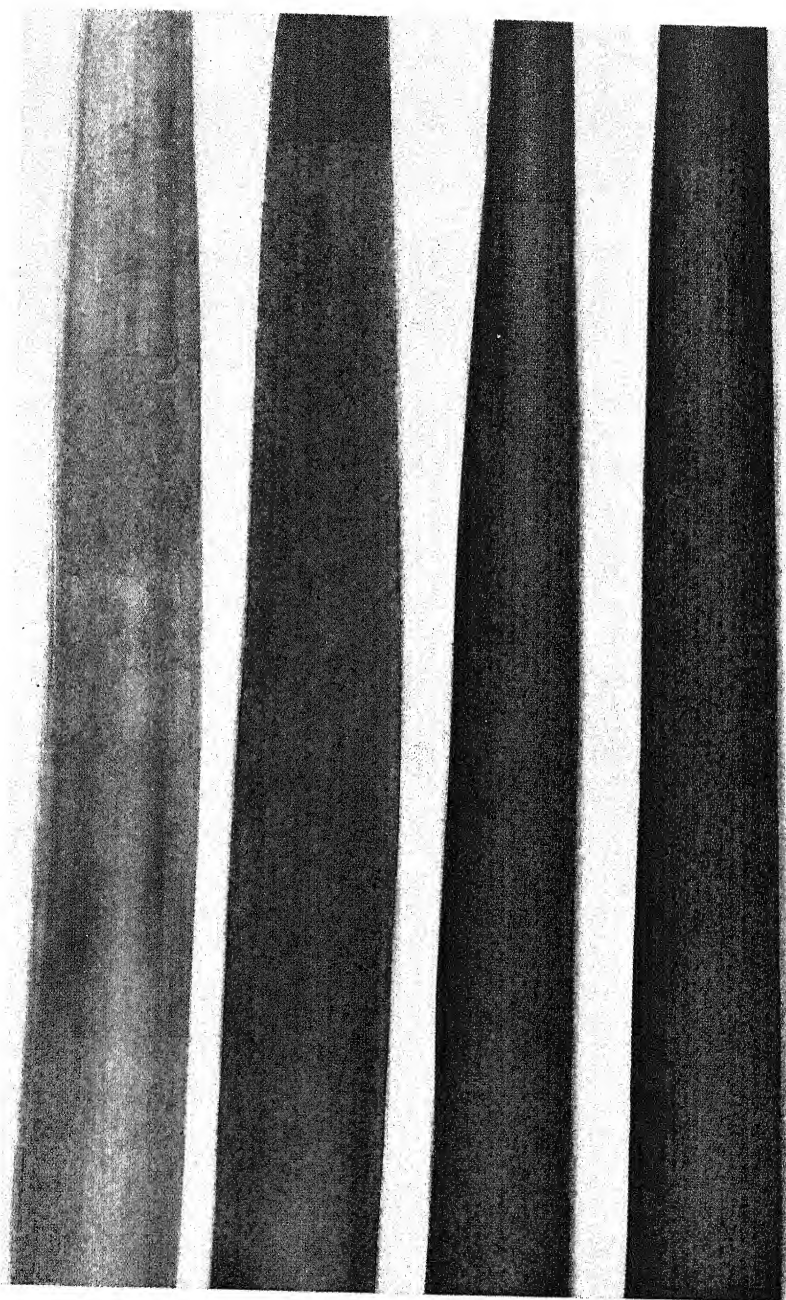
NO. 0 COLOR: YELLOW.—This region in the color scale is represented by senescent leaves or senescent areas of leaves. They are yellow or yellowish brown and practically free of any pronounced shade of green. The starch content of such leaves is variable, and it is doubtful whether these leaves contribute materially to carbohydrate metabolism.

NO. 1 COLOR: YELLOW-GREEN.—The median region of this color range is much less green than no. 2. It is decidedly yellowish and characteristic of leaves much exposed to sunlight. Leaves in this color range contain starch to within 75–100 per cent of capacity. Leaves may be injured by excessive sunlight or very high temperature, or both, and exhibit shades of greenish yellow similar to no. 1 color, but can be easily distinguished by characteristic uneven coloring and internally by disorganization of chloroplasts (8).

NO. 2 COLOR: OLIVE-GREEN.—The median region of this color range is olive-green. The upper whorls of leaves often exhibit this color as the plant matures. This is particularly noticeable in a sunny season or location, unless the plants are very heavily fertilized with nitrogen, or closely planted, or both. Starch reserves are within 50–75 per cent of capacity in leaves of this class.

NO. 3 COLOR: BLACKISH-GREEN.—The median region of this color range is very dark green. In much shaded, lower leaves it reaches an almost black-green. It is typical of plants under growing conditions when abundant nitrogen has been supplied or when subjected to relatively cloudy weather, or both. The starch content of leaves of this color is 25–50 per cent of capacity.

RECORDS OF LEAF COLOR.—Recording the color of the leaves is considerably simplified by the fact that there are usually rather definite zones of greenness. Invariably the lower leaves are darker green, midway along the stem the leaves are intermediate in greenness, while the upper whorls are comparatively less green. For each of the ten plants selected along the length of the two beds there is recorded for the plant as a whole the percentages of the leaf area that on the upper surface may exhibit one or more of the classes of color shown in figure 7, very young



0

1

2

3

FIG. 7.—Median region of four ranges of color of pineapple leaves. From left to right: no. 0, yellow; no. 1, yellow-green; no. 2, olive-green; and no. 3, "black"-green. With reference to maximum possible fruit production, no matter how low or how high nitrate reserves may be, carbohydrate—rather than nitrate—deficiency is indicated and limits yield if in warm sunny location there is less than 15 per cent and in very cool or cloudy location 40 per cent.

leaves and proximal and distal portions of older leaves being excepted, as already noted. The sum of the percentage of each color class equals 100 for each plant. Results are reported as averages of ten plants. Independent records by men trained in this work seldom vary by more than about 3 per cent in the color average of the same lot of ten plants. Very small differences in percentage of no. 1 color, yellow-green, are correlated with striking differences in fruit production, as will be later shown.

LEAF-COLOR INDEX.—Together with other determinations as a guide to nitrogen requirements, the percentage of no. 1 color stands out as highly significant in many experiments, probably because the upper leaves—relatively high in percentage of such color—are more active than other plant parts in nitrate reduction (24, 25). Thus, for certain purposes, the respective color values are kept segregated. On the other hand, a weighted leaf-color index of degree of greenness gives an expression of plant condition that is intimately associated with quality of fruit. The weighted leaf-color index is determined by multiplying the percentage of no. 1 color by one, the percentage of no. 2 color by two, and the percentage of no. 3 color by three. The results are added and the sum divided by three. The resultant figure is the leaf-color index.

RED PIGMENT.—The percentage of bluish-red anthocyanin pigment sometimes displayed on the upper surface of pineapple leaves may be recorded, but the degree of pigmentation commonly fluctuates too rapidly to be a useful index for most purposes. For instance, a change in air temperature, if it persists for a day or two, usually is associated with rather striking changes in anthocyanin content; low temperatures are followed by an increase if other factors remain essentially the same. Likewise, plants subjected to serious deficiency are commonly highly pigmented. Both these environmental influences, together with inadequate nitrate reserve, are often correlated with a comparatively high sugar content in the leaves. Results in harmony with these have already been reported by MAGNESS (12) and others for apple trees. Under all observed conditions, however, the ratio of starch to sugars is proportionately high as determined macrochemically when starch and total sugars are expressed as dextrose.

LEAF NITRATE-NITROGEN.—The leaf shown at the center of figure 6 illustrates the precise stage of growth of leaves employed for nitrate analysis. They are almost fully expanded, except for the lateral points at the base which, in a fully mature leaf approaching senescence, form a much more acute angle, and in an immature leaf form approximately a right angle. It is thus simple always to select exactly comparable leaves for analysis, regardless of environmental influences or whether the plant has been growing for 3 months or a year. After blossom buds appear, all leaves on the original stem become mature and no further analyses are made for nitrate. Experimental trials have shown that nitrogen fertilization of

pineapple, after blossom buds develop, is without material influence upon the "mother plant" or fruit. These results are in harmony with the responses of some other monocotyledonous plants, such as oats, as has been shown by WILLIAMS (33).

For each of the ten plants used as the basis of records, the leaf as just described is pulled out for nitrate analysis. It breaks off easily and cleanly near the point of attachment to the stem. In this region the white basal tissue is semi-meristematic. It is comparatively homogeneous and contains practically no lignified elements. In several lots of leaves the moisture content in the middle third of the white basal tissue employed for nitrate analysis varied from 89 to 92 per cent. This was true even though the relatively mature portion of the leaf had in some lots a severe water deficit in the specialized water-storage tissue, while others were filled to capacity with stored water, and still others were only partially filled.

Leaves may be harvested for analysis at any time between 8:00 A.M. and 4:00 P.M. At least, tests of comparable plants of several lots indicated no material variation in nitrate content in the leaf owing to the hour of sampling. The middle third of the white basal tissue of the ten leaves from as many plants is minced, mixed, and an aliquot homogenized in water in a small, electrically driven mixer. The amount of nitrate in the clear filtrate, entirely free of any noticeable color, is determined by the phenoldisulphonic acid method (1) and is expressed as percentage of nitrate nitrogen. Re-sampling of any plot or bed seldom shows, on the same or following day, a departure from the original results by more than plus or minus 0.003 per cent of nitrate nitrogen.

ROLE OF NITRATE IN THE PLANT. —Leaf-nitrate values, as obtained month after month on tissues strictly comparable physiologically, are directly correlated with the nitrate content of the massive stem of the pineapple plant which may have up to half or more of its total nitrogen content in the form of nitrate. The green portion of the leaf contains little or no nitrate, except under conditions preventing its reduction (18). Nitrate does not directly affect the growth responses of plants materially. There must first occur nitrate reduction, oxidation of sugars, and organic nitrogen synthesis; but a sufficiently active rate of synthesis of amino acids and proteins in many plants does not occur unless the tissues concerned contain a liberal reserve of nitrate (18). Pineapple is apparently no exception. It is also like most other plants in that it does not store ammonium (24, 25), even when ammonium sulphate is practically the only nitrogenous fertilizer employed. Also, under field conditions ammonium is rapidly oxidized to nitrate in the soil. While the pineapple plant can freely absorb and elaborate ammonium (24, 25) under field conditions, it seldom—if ever—has an opportunity to do so for a significant length of time.

While any cell constituent may well be considered as exerting some influence on the development of the plant, nitrate is obviously not an essential form of nitrogen,

for—by employing an ammonium salt as the sole external source of nitrogen (18)—many species of nitrate-storing plants, including pineapple (24, 25), have been grown to maturity and have produced abundant fruit when grown in water cultures with the nutrient solution and the plant itself almost or entirely free of nitrate.

LEAF POTASH AND LEAF PHOSPHORUS.—There are important interdependent relationships between nitrate, phosphorus, and potash in the nutrition of the pineapple plant that will not be discussed in detail in this paper. For determination of total potash and total phosphorus, however, aliquots of the same white basal leaf tissue employed for nitrate analyses are ashed and official methods followed (1).

GROWTH INDEX.—This is the weekly rate of increase in length of young leaves, initially 20 cm. long, selected at monthly intervals. The same lots of plants are always employed, care being taken to select representative locations in the field.

IRON DEFICIENCY.—Plants are recorded as being deficient in iron on the basis of the percentage of the leaf area, if any, which exhibits chlorotic mottled areas characteristic of such deficiency. In productive commercial pineapple culture, however, iron deficiency seldom is allowed to persist long enough to be of practical significance, the condition being remedied by spraying with iron sulphate.

ZINC DEFICIENCY.—On the basis of the percentage of leaf area exhibiting characteristic mottled spots or blisters, plants are recorded as being deficient in zinc. Such deficiency is easily prevented by infrequent spraying with zinc sulphate, however, and at present is seldom found in significant degree in commercial fields.

LEAF-WATER DEFICIENCY.—The pineapple plant may not only store very high reserves of starch and nitrate, but in addition the leaves possess specialized water-storage tissue. Macroscopically this is apparent as a clear translucent layer beneath the upper epidermis. Its development proximately keeps pace with differentiation of other leaf tissues. The white semi-meristematic basal tissue of leaves employed for nitrate analysis, being largely undifferentiated, does not include specialized water-storage cells. When the ten leaves from as many plants are collected for nitrate analysis, they are arranged in a bundle with their basal ends placed one on top of another. The bundle of leaves is then cut crosswise, one-third of the distance up from the base, where water-storage cells are fully differentiated. The ratio of the thickness of water-storage tissue to that of chlorophyll-containing tissue, when viewed in transverse section, is then recorded. If the thickness of water-storage tissue is less than that indicated by the following ratios for the appropriate age, it is so reported on a percentage basis.

STAGE OF GROWTH	WATER- STORAGE TISSUE	NONWATER- STORAGE TISSUE
First 10 months.....	1	2
Eleven months to dried flowers.....	2	3
After dried flowers.....	1	1

SUNSHINE RECORDS.—For this purpose, two soil or distance thermographs are employed, the sensitive element of which is a cylindrical copper bulb 12 inches long and about 1 inch in diameter. The bulb of one instrument is placed in a conventional louvered weather shelter; the other bulb is painted black and exposed to full sunlight in a horizontal position, with the length of the bulb in a north-south direction. The difference in temperature between the bulb in the weather shelter and that exposed to sunlight is recorded on the charts of the two instruments. This difference is converted to terms of foot candles on the basis of comparative tests with several instruments for recording sunlight. Results are expressed as the average weekly light rate in foot candles from sunrise to sunset, employing formulas developed by FARDEN (5). These instruments are sufficiently sensitive for the purpose employed. In different locations and seasons, the average weekly light rate varies from less than 2000 to about 8000 foot candles.

AVERAGE 2-HOURLY AIR TEMPERATURE.—In its relation to the nutrition of the pineapple plant, the most consistently useful, single expression of air temperature has been the average 2-hourly day-and-night records from thermographs. On the other hand, it is often important to segregate the average day and night temperatures, for two fields may have the same average 24-hour temperature, but if the nights are relatively warm, the plants in that field will be much lower in carbohydrate reserves than in the other if conditions in both locations—including sunlight—are otherwise similar. Past weather records for a location are of considerable local value in connection with the nitrogen nutrition of pineapple, for they have a bearing upon what may reasonably be expected from a fertilizer application to plants with known root systems and content of starch and nitrate. Plants alike in size and quality, and with adequate soil moisture, will not give the same responses in fruit production to the same fertilizer treatment if there follow different conditions of air temperature and sunlight. These and related factors will be discussed later.

FRUIT EXPOSURE.—About a week before fruit is to be harvested, the various plant records are taken as described, except analyses of leaves for nitrate, potassium, and phosphorus. At that time there is also recorded the percentage of total possible exposure of the fruit to direct sunlight. This might seem a superfluous determination, but fruit temperature in large measure determines acidity and texture of the flesh of the fruit. In turn, fruit temperature varies with that of the air, but particularly with the amount of sunshine and the degree of exposure to it. For instance, if the average fruit temperature during the week prior to picking is high because of a high light rate or a high percentage of exposure to sunlight, or both, the fruit will be very low in acidity. Conversely, low fruit temperature makes for high acidity. The explanation may lie in the well-recognized effects of temperature on rate of respiration and concomitant decarboxylation (26).

That air temperature alone is no index of fruit temperature is apparent from the fact that on sunny days a fruit with about 100 per cent of possible exposure to sunlight may attain a flesh temperature of 130° F., with a maximum air temperature of only 73° F. Formulas, not given here, have been worked out for computing fruit temperature on the basis of light rate, air temperature, and percentage of exposure to sunlight. Excepting the crown leaves of the fruit itself, exposure is considered 100 per cent when no leaves at any time of the day shade the sides of the fruit. Exposure is recorded as zero if the sides of the fruit are not exposed to direct sunlight at any time of the day. By employing suitable photographs as standards of comparison for different degrees of exposure between these two extremes, reliable records are obtained.

The temperature of the flesh of the fruit has the influences noted; but in relation to sugar content, the average air temperature—taken at 2-hour intervals from thermograph charts during the week prior to harvest—is a dominant factor. The plant does not store starch or other carbohydrate reserves in quantity in unripe fruit, but shortly before ripening there is a rapid influx of sugar from the vegetative organs to the fruit, the rate apparently being determined in large part by air temperature. Under adverse conditions, when the average air temperature for 2-hour intervals during the week prior to picking is low (68° F.), plants high in starch reserves (in that they produce fruits relatively high in sugars) have a decided advantage over plants comparatively low in carbohydrates. This advantage is negligible, however, under favorable intermediate conditions of 69° – 75° F. average air temperature during the week prior to picking.

Discussion of results

The pineapple plants of these experiments were all abundantly fruitful, in the sense that they would be placed in KRAUS and KRAYBILL's class III (10). In no case were nitrate or carbohydrates so deficient that lack of either was associated with nonfruitfulness. Nor were any of the plants more extreme in type of growth and composition than have often been found in the past in fields supplied with a predetermined schedule^{*} of nitrogen applications. Thus this report deals with a relatively narrow range of fruitful plant types, with differences in degree of nitrate—or carbohydrate—deficiency that are comparatively small as compared with the extremes encompassed by KRAUS and KRAYBILL's four classes. At the time of differentiation, however, even a seemingly slight deficiency of nitrate as related to carbohydrates was correlated with marked decreases in yields of fruit if not rather

^{*} A schedule usually decided upon before a field is planted. It gives the dates when nitrogen is to be applied and the amounts. It is largely derived by averaging the results of several years of conventional fertilizer tests employing six replications of each treatment, analyzed each year for statistical significance, with results varying in different years with the weather, to the extent that there may be a 75 per cent difference in total amount of nitrogen required in successive years.

promptly adjusted. These deficiencies, quantitatively measured in the growing plant, have been found remarkably accurate indices of the amounts of nitrogenous fertilizer that may be required at any time, in relation to carbohydrates, for greatest fruit production.

To eliminate as many limiting factors as possible, other than the known levels of nitrate—or carbohydrate—deficiency being tested, in their relationship to production of fruit, all the experiments were conducted in fields where it has been demonstrated that neither potassium nor phosphorus was lacking. When limiting factors appeared, such as deficiency of iron or zinc, they were promptly corrected by applications of sprays containing these elements. Experimental results from areas lowest in sunshine are not included in this report, for such fields are typically in the rainy mountain sections where the soil, owing at least in part to leaching, is usually deficient in potassium and phosphorus.

Unless otherwise indicated, it should be understood that during the experimental periods under consideration, the percentage of white root tips was sufficient for adequate absorption of water and other nutrients, that root anchorage was 100 pounds or more, and that the soil moisture was above the wilting percentage. After the plants were a few months old, ammonium sulphate, when employed, was applied as usual in the lower leaf axils, and therefore did not become available until there had been sufficient rain to dissolve and carry it to the roots. Accordingly, there is indicated in this report not only the actual date of application of fertilizer but the proximate date when it was made available by rain. Weather records, for the most part, are reported only when they appear to be pertinent. The detailed reports of climate, while of local value, are secondary to the theme of this paper—nitrate and carbohydrate reserves as correlated with yield of fruit. The pineapple plant itself is, in part, an expression of these relationships and a reflection of favorable and unfavorable environmental influences more exact than can be derived from records of environment alone.

The experimental results that follow indicate considerable differences in yields of fruit from plants essentially similar in content of nitrate and starch. Differences in yields were often correlated with differences in size of the plants at the time flower-bud primordia developed. Plant size is influenced by many factors, such as number of plants per acre; number of months of vegetative growth, which varies materially with the season of planting; length of time from planting to emergence of roots, which varies with the amount of rain as well as with the carbohydrate reserves in the planting material, and so on. On the other hand, the temperature during the period from appearance of flower buds until the fruit is harvested is of extreme importance in relation to size of fruit. For example, a plant weighing 16 pounds, deficient in neither nitrate nor carbohydrates, may produce a fruit weighing less than 5 pounds if the weather is cool during the 5–6 months from the first

appearance of blossom buds until the fruit is ripe. In contrast, a plant of equally desirable chemical composition, weighing less than half as much, may produce a fruit weighing more than 6 pounds if the temperature conditions are favorable during this same period. Exact quantitative data of this type are available but are not conveniently included in this report.

Figure 8 shows, for various stages of growth, the proximate maximum percentage of nitrate-nitrogen found in the white basal tissue of the leaf employed for analysis. Occasionally nitrate may attain levels slightly higher than those indi-

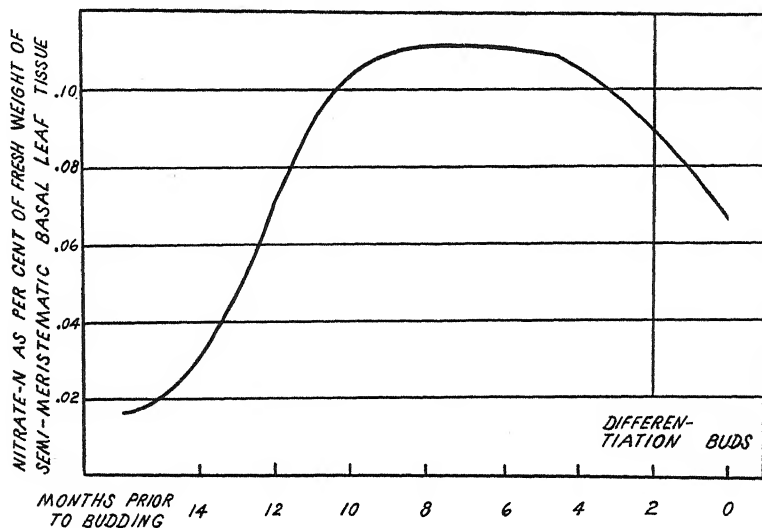


FIG. 8.—Proximate maximum percentages of leaf nitrate-N attainable at different stages of growth. With reference to greatest possible yields, maximum nitrate is desirable only if plants contain adequate carbohydrate reserves, as indicated in warm sunny location by not less than about 15 per cent and in very cool or cloudy location by not less than about 30 per cent no. 1 yellow-green leaf color (cf. fig. 7).

cated, and frequently—if reduction is limited by any factor—will become abnormally high. But it has yet to be demonstrated that increases in yield of fruit have been brought about by raising the percentages of nitrate, for the different periods of plant development, higher than those shown in figure 8. On the contrary, there is evidence to indicate that opportunity for carbohydrate synthesis and accumulation must be exceptional before it becomes desirable to attempt continually to maintain leaf nitrate at the essentially maximum levels indicated in figure 8. Rather it is proposed to show that when nitrogen is added to plants deficient in starch, as indicated by a percentage of no. 1 leaf color (fig. 7) lower than about 15 per cent, yields will not be significantly increased even if leaf-nitrate values are very low for the stage of growth concerned as compared with those shown in figure

8, and even if the nitrate content of the plant is increased. Exceptions to this general rule apparently occur when temperature and sunlight following nitrogen fertilization are unusually favorable for carbohydrate accumulation. In contrast, in locations or seasons of the years, when it is known from past records that weather conditions following a contemplated application of nitrogen to a field are not likely to favor carbohydrate storage (as in cloudy mountain areas not considered in this report), a materially greater percentage of no. 1 color—about 30 per cent—is apparently required before material increases in fruit production occur owing to added nitrogen or absorbed nitrate.

As already pointed out, after blossom buds are well advanced (fig. 4), fertilization with nitrogen has no measurable influence on yield, even in plants extremely deficient in nitrate and containing abundant carbohydrate reserves. At approximately the time of floral differentiation, however, about 2 months before blossom buds become apparent or even a few weeks earlier, plants low in nitrate in relation to starch reserve often respond to added nitrogen with marked increase in production of fruit, if there are no other limiting factors. That the pineapple plant is particularly responsive at this stage of growth may be owing, at least in part, to the fact that it has approximately reached its maximum size, as vegetative expansion of the original plant essentially ceases when the determinate flower bud appears. Thus the total protoplasmic volume available for metabolizing nitrate is probably greater than at any earlier period.

NITRATE DEFICIENCY AT TIME OF FLOWER-BUD DIFFERENTIATION.—It is convenient to consider first the effects on relative yield of fruit of different degrees of nitrate deficiency, maintained for a short period of time, definitely delimited initially by given dates of plant analysis at a little before floral differentiation and terminated finally by emergence of buds (fig. 4), when measurable influences of nitrate on production of fruit cease. It is emphasized that nitrate is not considered deficient, no matter how much lower it may be than the percentage values given in figure 8, for lack of it does not usually limit yields when coupled with deficiency of carbohydrates, as indicated by less than about 15 per cent no. 1 yellow-green leaf color (fig. 7).

From July to the end of August (table 1) the plants decreased in nitrate from 0.090 to 0.063 per cent. In the relatively warm location where they were growing there is a light rate during the summer and fall of typically about 5000 foot candles. Higher nitrate reserves were evidently desirable, for there was no evidence of carbohydrate deficiency, no. 1 leaf color being 21 per cent in August. These nitrate-deficient plants were then fertilized with nitrogen, at that time only in the case of the B and D plots. About a month later definitely higher nitrate reserves were found than in the plants of the A and C series. The D group, with no more nitrogen added (after 120 pounds was applied in September), were thereafter con-

sistently higher in nitrate than the A group, which was unfertilized during the experimental period. Although the D plots were relatively low in carbohydrates, as compared with the A, they had at no time less than 16 per cent no. 1 leaf color and at the time of differentiation displayed 24 per cent. Correlated with these conditions, the D group outyielded the A by more than 3 tons of fruit per acre. That the C plots, not supplied additional nitrogen until late October, yielded slightly less fruit than the D was owing in large part to the fact that the increase in nitrate reserves was not attained until a little later than in the plants of the D plots. As shown in table 1, the plants of the B group were provided (in two applications)

TABLE 1

EXPERIMENT 29 F 3: FIELD 5518, PLANTED NOVEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

LOT	PRI- OR NI- TRO- GEN (LB.)	7/26/40		8/30/40		9/16/40		10/25/40		10/25/40		12/14/40		12/19/40		12/30/40		TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. 1	NO ₃	No. 1	(8/16) NI- TRO- GEN (LB.)*	NO ₃	No. 1	(10/8) NI- TRO- GEN (LB.)*	DIFFER- ENTIA- TION†		(11/13) NI- TRO- GEN (LB.)*	NO ₃	No. 1	TO- TAL	PLUS OR MINUS B	RE- QUIRED FOR ODDS 19:1				
																			NO ₃	No. 1	
A.	200	.030	23	.063	21	None	.034	33	None	.051	47	None	.037	51	200	28.88	-3.74	0.71			
B.	200	.030	23	.063	21	80	.052	21	None	.076	17	80	.073	24	360	32.62	0.71			
C.	200	.090	23	.063	21	None	.033	28	80	.070	28	None	.059	27	280	31.42	-1.20	0.71			
D.	200	.090	23	.063	21	120	.057	16	None	.063	24	None	.053	27	320	31.91	-0.71	0.71			

* Ammonium sulphate applied in lower leaf axils on dates given in parentheses, but because of lack of sufficient rain to dissolve the fertilizer and carry it to the roots, it was not available until the proximate later date shown.

† Budding occurred about the middle of February.

with a total of 40 pounds more nitrogen than the D group, were consistently higher in nitrate reserves, were at no time deficient in carbohydrates (as 17 per cent was the lowest value for no. 1 leaf color), and—just within the limits of odds of 19:1—they significantly outyielded the D series by 0.71 tons per acre. That the gain in yield was only slightly above that of the D plants was probably owing to the fact that at time of differentiation, when the second application of nitrogen became available on December 14, the plants were nearly filled to capacity with nitrate (fig. 8) and displayed 17 per cent no. 1 color, which is only a little above the arbitrary line of carbohydrate deficiency—considered to be about 15 per cent. Light rate and temperature were such that this did not result in carbohydrate deficiency, however, for even though the plants were essentially filled to capacity with nitrate, they exhibited 24 per cent no. 1 leaf color at the end of December.

The results summarized in tables 1 and 2 for the two experiments in field 5518 show that considerable differences occurred in the relative yields of plants of com-

parable composition with similar nitrogen fertilization. The sites of the experiments, although in the same field, were well over a $\frac{1}{2}$ mile apart, and while sunshine, temperature, and rainfall were much the same, comparatively high wind velocity was probably partly responsible for relatively limited growth, in case of experiment 29 F 3. (Experimental trials with pineapple plants growing in aliquots of the same soil in asphalt-painted iron containers, furnished with drainage outlets, and abundantly supplied with water and fertilizer, have shown that even moderate wind velocity, if long continued, may decrease the size of plants by as much as 25 per cent as compared with others not exposed to wind but under the same conditions of temperature and sunlight.) Nevertheless, in spite of differences

TABLE 2

EXPERIMENT 29 F 3A: FIELD 5518, PLANTED DECEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NI- TRO- GEN (LB.)	11/18/40		11/19/40	12/14/40		12/30/40		2/14/41	To- tal NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. 1	NITRO- GEN (LB.)*	DIFFERENTIA- TION		NO ₃	No. 1	(BUDS) No. 1		TOTAL	PLUS OR MINUS D	RE- QUIRED FOR ODDS 10:1
					NO ₃	No. 1							
A....	285	.054	25	60	.070	25	.066	27	15	345	35.54	+2.00	0.64
B....	285	.054	25	80	.073	26	.070	25	15	365	35.40	+1.86	0.64
C....	285	.054	25	100	.074	25	.071	24	12	385	36.33	+2.79	0.64
D....	285	.054	25	None	.064	33	.053	36	30	285	33.54	0.64

* Ammonium sulphate applied in lower leaf axils November 13, but there was not sufficient rain to dissolve and carry it to the roots until November 19.

inherent in the two experimental sites, in both experiments—following diminution in degree of nitrate deficiency with different nitrogen treatments—the yields were increased. When ammonium sulphate was added to all except the D plots, on November 18 (table 2), the plants had a leaf-nitrate content of only 0.054 per cent. This was a low value in relation to the liberal starch reserve indicated by 25 per cent no. 1 leaf color. About a month following fertilization, however, plots A, B, and C increased materially in nitrate as compared with plots D. As a result, the plants of the D plots were outyielded by about 2 tons of fruit per acre. Limited fruit production in the D plots was unquestionably correlated with a nitrate reserve definitely deficient in relation to the high starch content, indicated by over 30 per cent no. 1 leaf color in December. That the plants—especially of the C plots that produced 2.79 tons more fruit per acre than the D—absorbed approximately the maximum amount of nitrate they could metabolize without incurring carbohydrate deficiency is indicated by only 12–15 per cent no. 1 color at budding.

In July, as shown in table 3, the plants in field 5530 were filled nearly to capacity with nitrate (fig. 8) and on the borderline of carbohydrate deficiency, as indicated by only 14 per cent no. 1 color. The decrease in nitrate from 0.097 to 0.070 per cent in only one month, from July to August, might be considered too rapid were it not for the fact that carbohydrate reserves were low, as indicated by slightly less than 15 per cent no. 1 leaf color in both months. These responses were associated, however, with an average 2-hourly temperature for both months of more than 72° but with a light rate of less than 4000 foot candles. Nevertheless, by the end of October, with no nitrogen added to the A plots, leaf nitrate was 0.030 per cent, too low in relation to the abundant carbohydrate reserves indicated by 25 per cent no. 1 leaf color. In contrast, plots B, C, and D, supplied with nitrogen on the dates

TABLE 3

EXPERIMENT 29 F 3: FIELD 5530, PLANTED SEPTEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NITRO- GEN (LB.)	7/8/40		18/13/40		8/24/40	10/8/40	10/23/40		11/13/40	12/12/40		12/27/40	TO- TAL NITRO- GEN (LB.)	FRUIT (TONS PER ACRE)			
		NO ₃	No. I	NO ₃	No. I	NITRO- GEN (LB.)	NITRO- GEN (LB.)	NO ₃	No. I	NITRO- GEN (LB.)	DIFFER- ENTIA- TION*		NO ₃		No. I	TOTAL	PLUS OR MIN- US A	RE- QUIRED FOR ODDS 19:1
											NO ₃	No. I						
A	260	.097	14	.070	12	None	None	.030	25	None	.039	36	.039	32	260	32.60	0.85
B.....	260	.097	14	.070	12	80	None	.038	16	80	.062	15	.072	14	420	35.69	+3.09	0.85
C.....	260	.097	14	.070	12	None	80	.030	24	None	.059	23	.059	19	340	35.15	+2.55	0.85
D.....	260	.097	14	.070	12	120	None	.045	11	None	.054	18	.060	16	380	35.69	+3.09	0.85

* Budding occurred about the middle of February.

and in the amounts given in table 3, were in December materially higher in nitrate than the A plots, were lower in carbohydrates, and outyielded the nitrate-deficient A plots by about 3 tons of fruit per acre. The second application of nitrogen to the B plots did not increase yields over the D: While the difference in total amount of nitrogen added to B and D plots was only 40 pounds, the second fertilization of the B plots was made when the plants exhibited only 16 per cent no. 1 leaf color, and—while nitrate was subsequently nearly doubled—carbohydrates were continually at a very low level, for no. 1 color remained at 14–15 per cent. It is highly probable that lack of carbohydrates limited reduction of nitrate and new protein synthesis.

The results reported in table 4 show that on November 18 the plants in field 5530 contained only about half the maximum possible nitrate content (fig. 8). Nevertheless, 0.054 per cent leaf nitrate was clearly adequate, for carbohydrates associated with the climatic conditions already noted were low, as indicated by

16 per cent no. 1 leaf color. If past weather records (usually indicative of comparatively favorable conditions for carbohydrate accumulation in field 5530) are considered, and the fact that there were yet about 4 months before budding—during

TABLE 4

EXPERIMENT 29 F 3A: FIELD 5530, PLANTED SEPTEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NI- TRO- GEN (LB.)	11/18/40		11/19/40	12/13/40		12/26/40		2/14/41	TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. 1	NITRO- GEN (LB.)*	DIFFERENTIA- TION		NO ₃	No. 1	BUD No. 1		TOTAL	PLUS OR MINUS D	RE- QUIRED FOR ODDS 19:1
					NO ₃	No. 1							
A....	340	.054	16	60	.063	14	.073	18	21	400	35.55	+0.92	0.62
B....	340	.054	16	80	.071	20	.086	16	17	420	35.86	+1.23	0.62
C....	340	.054	16	100	.063	18	.079	19	22	440	36.25	+1.62	0.62
D....	340	.054	16	None	.054	23	.056	22	35	340	34.63	0.62

* Ammonium sulphate applied in lower leaf axils on November 13, but there was not sufficient rain to dissolve and carry it to the roots until November 19.

TABLE 5

EXPERIMENT 29 F 1: FIELD 5510, PLANTED JANUARY, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRI-OR NITRO-GEN	8/28/39		9/15/39	10/7/39		11/27/39		12/25/39	1/4/40		2/12/40		TO-TAL NITRO-GEN (LB.)	FRUIT (TONS PER ACRE)			
		NO ₃	NO. 1	NITRO-GEN (LB.)	NO ₃	NO. 1	PRE-DIF-FERENTIA-TION		NITRO-GEN (LB.)*	NO ₃	NO. 1	BUDS			TOTAL	PLUS OR MI-NUS D	RE-QUIRED FOR ODDS 19:1	
												NO ₃	NO. 1					NO ₃
A....	285	.102	17	80	.091	24	.086	21	None	.071	19	.045	17	365	34.67	+0.63	0.92	
B....	285	.102	17	None	.087	25	.071	30	80	.058	25	.056	16	365	34.74	+0.70	0.92	
C....	285	.102	17	80	.091	24	.086	21	80	.074	19	.055	13	445	36.29	+2.25	0.92	
D....	285	.102	17	None	.087	25	.071	30	None	.058	28	.039	28	285	34.04	0.92	

* Ammonium sulphate applied in lower leaf axils on December 6, but there was not sufficient rain to dissolve and carry it to the roots until the last of the month.

which period nitrate might become deficient—additional application of nitrogen would be required, particularly since analyses prior to those of November 18 showed that nitrate was decreasing rapidly. Following application of nitrogen to plots A, B, and C, there was (table 4) an increase in leaf nitrate that by December 26 was nearly up to maximum for the stage of growth (fig. 8). This was coupled

with maintenance of adequate starch reserves, as indicated by more than 15 per cent no. 1 color in all plots by the end of December and at budding in February. Associated with these responses was a light rate from December through February of more than 5000 foot candles. Correlated with little or no deficiency of either nitrate or carbohydrates in plots A, B, and C, the plants significantly outyielded those of the D group which were clearly deficient in nitrate in relation to their comparatively high starch reserves.

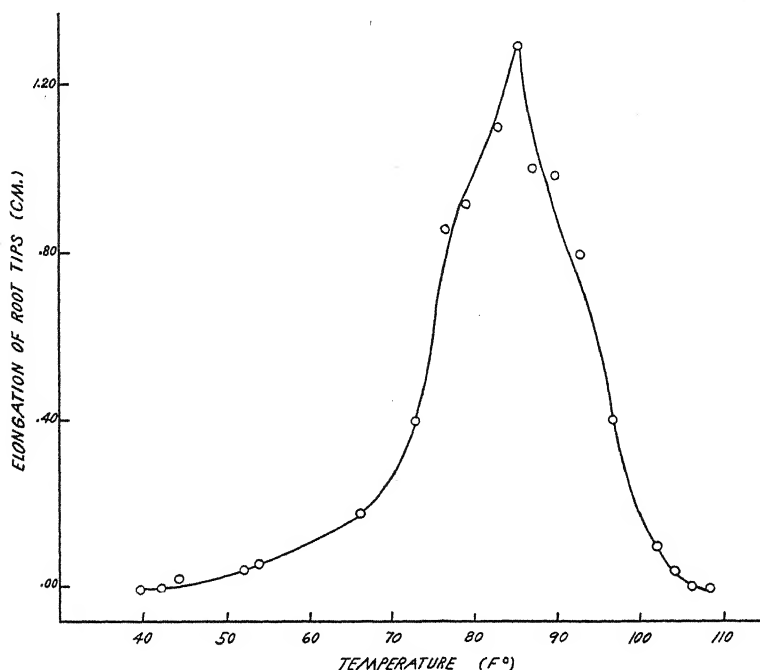


FIG. 9.—Comparative elongation of pineapple roots in tap water as affected by temperature. Variety Smooth Cayenne. Data from WATANABE (30). Roots of standard length measured before and after 48 hours at temperatures indicated. Note limited growth below 68° and above 98°.

The foregoing report on relative yields associated with various degrees of nitrate deficiency, in plants containing sufficient reserves of carbohydrates, is characteristic of an enormous amount of experimental evidence obtained in many locations during a period of several years. There would seem to be little gained, however, by mere repetition of similar plant performances in other experiments. But table 5 includes, in addition to results similar to those already discussed, rather clear indications of the importance of continually maintaining adequate nitrate reserve in relation to carbohydrates the last 3 months before buds emerge. This is in contrast to earlier stages of growth when, as will be shown, plants may be per-

mitted to decrease materially in nitrate in relation to carbohydrates for 1-2 months with little or no effect on yields. Table 5, however, shows that this is far from the

EXP. 3/D (FD. 5477)

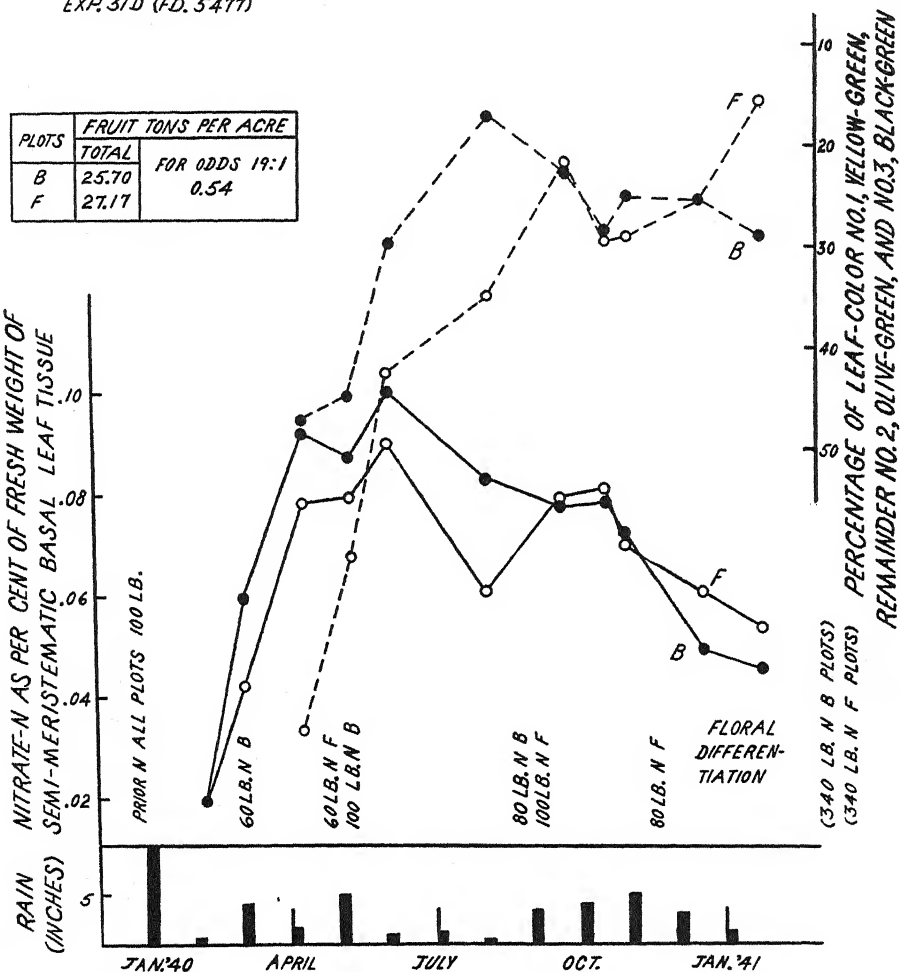


FIG. 10.*—With same total nitrogen fertilization, plots F outyielded plots B, demonstrating relative importance at floral differentiation of adequate nitrate in relation to no. 1 leaf color. Compare earlier months when plants of F series were nitrate deficient and those of B were not. At differentiation, plants of B were too low in nitrate for the high carbohydrate reserve indicated by 30 per cent no. 1 color.

* Figs. 10-14: broken lines, leaf color; solid lines, leaf nitrate.

case during the period just prior to budding, for the C plots—continually maintained with adequate nitrate reserves—gave a gain of over 2 tons of fruit per acre as compared with the D plants, which were extremely deficient in nitrate. This

was true even though prior to October, at initiation of the experiment, all the plants were filled to capacity (fig. 8) with nitrate, the leaf value being 0.102 per cent coupled with but 17 per cent no. 1 leaf color. In contrast to the high-yielding

EXP. 31D (FD.5530)

PLOTS	FRUIT TONS PER ACRE	
	TOTAL	FOR ODDS 19:1
B	34.23	0.78
F	35.79	

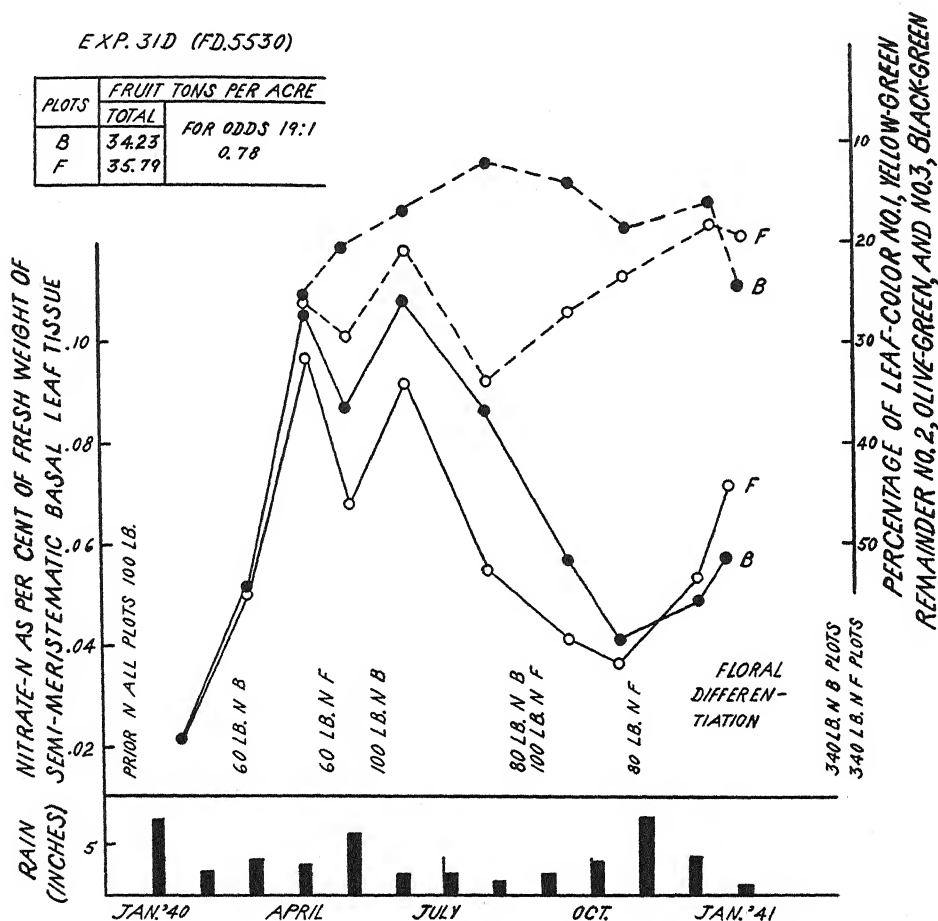


FIG. 11.—As in fig. 10, note relative importance at floral differentiation of avoiding nitrate deficiency in relation to carbohydrates (no. 1 color). Plots F, adequately supplied with nitrate at this period, out-yielded those of B that in relation to carbohydrates (no. 1 color) were deficient in nitrate at that time but highest in nitrate earlier. Note much higher nitrate reserves at differentiation than in field 5477 (fig. 10). Field 5530 is a relatively warm location. Greater nitrate absorption but carbohydrate deficiency did not occur, as even F plots were over 15 per cent no. 1 color at differentiation.

C plots, the plants of the A plots were deficient in nitrate in January and February, and the B plants were lacking in nitrate from November through December and at least the early part of January. The temporary elimination of nitrate deficiency in

the A and B plots by the application of 80 pounds of nitrogen, as indicated in table 5, did not give significant increases in yield. On the other hand, the C plots,

EXP. 31D (FD.5518)

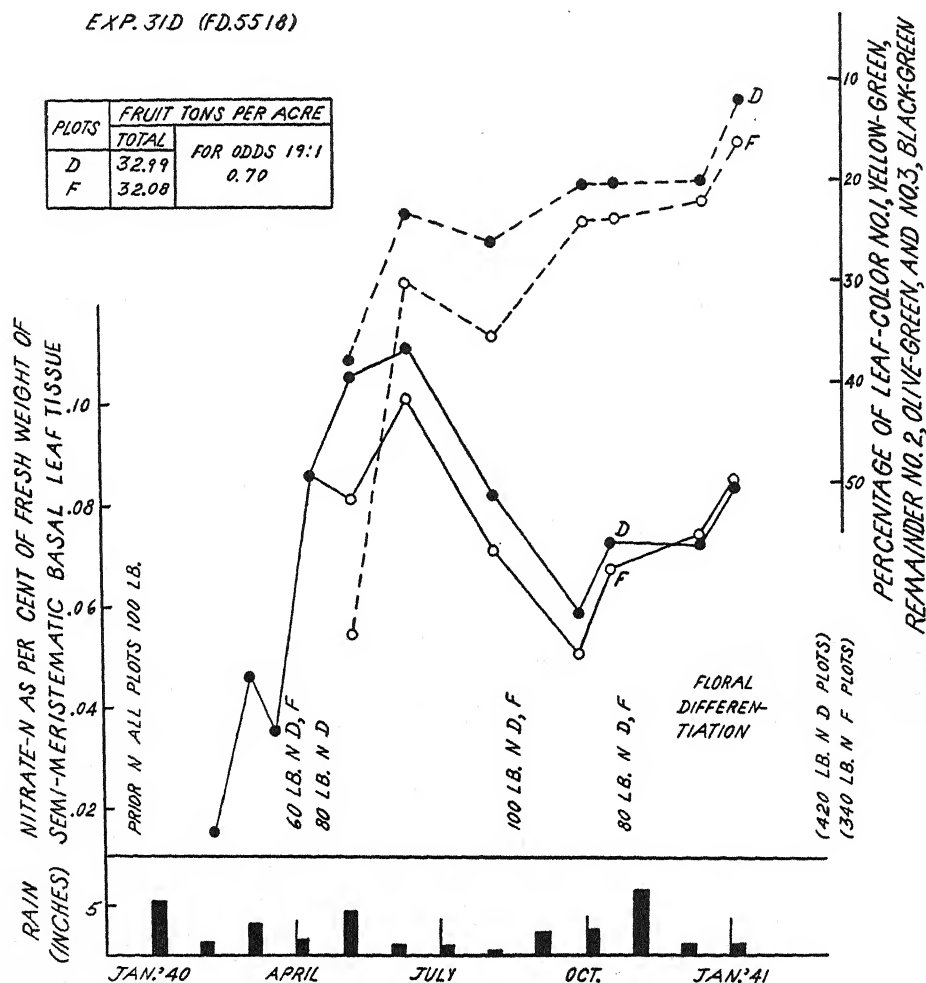


FIG. 12.—In contrast to figs. 10 and 11, at floral differentiation nitrate was not deficient in relation to carbohydrates in either plots D or F, for they approached the arbitrary point of carbohydrate deficiency, about 15 per cent no. 1 color. Earlier, F was deficient in nitrate in relation to no. 1 color. Accordingly F was significantly but not as greatly outyielded by D as would probably have been the case if nitrate had been low at differentiation instead of earlier.

that at no time were deficient in nitrate owing to the 160 pounds of nitrogen added, gave more than three times the gain of either A or B.

The time element was undoubtedly important in relation to these responses. As

nitrate is not directly essential for the growth of pineapple (24, 25) or probably any other green flowering plant (17, 18), it may well be asked why it is necessary to maintain any particular concentration of it in the pineapple plant as long as the

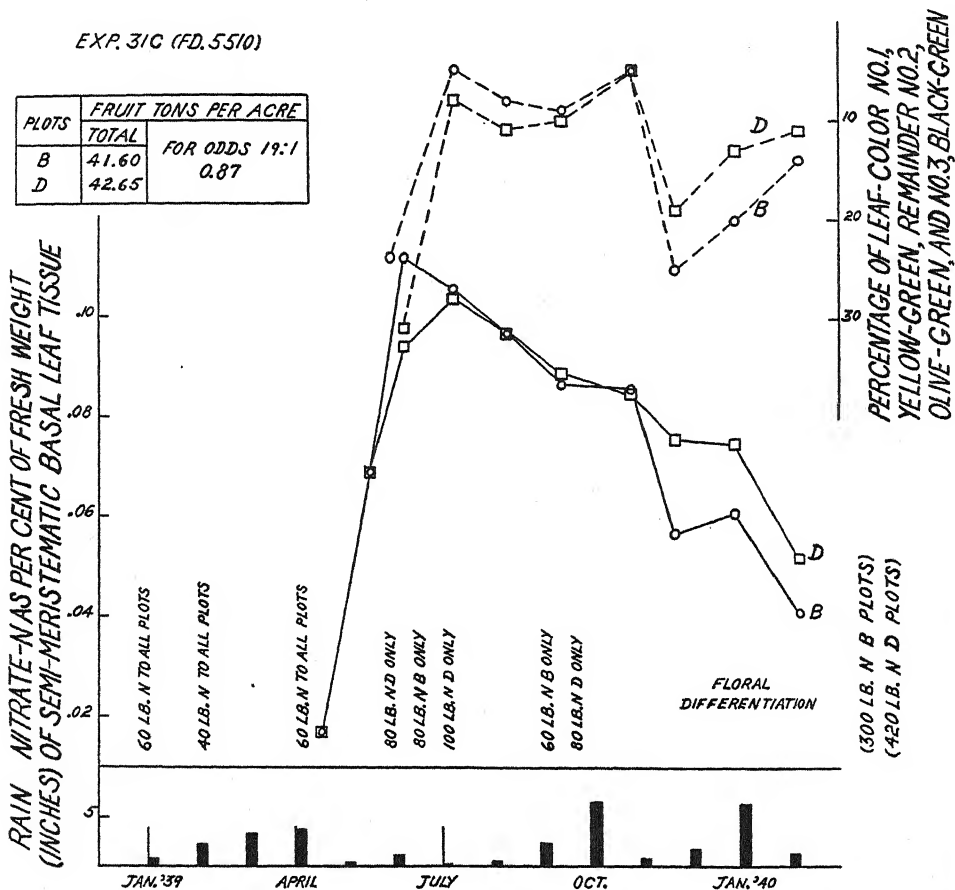


FIG. 13.—Unlike figs. 10–12, note that prior to floral differentiation (not at differentiation) plots D and B were about equally and adequately supplied with nitrate. Higher nitrate in midsummer could not have increased yields, no. 1 color being only 10 per cent, indicative of approaching carbohydrate deficiency. With higher carbohydrate reserves indicated in November by increase to about 20 per cent no. 1 color, B should have been higher in nitrate. It was accordingly outyielded by D, that in relation to carbohydrates was adequately supplied with nitrate.

reserve of nitrate is not completely exhausted. While the answer cannot be given, it would seem that nitrate in the plants at low concentration is not metabolized as efficiently, or freely, per unit of stored nitrate as when the concentration is higher, provided sufficient carbohydrates are available for the growth responses desired

and for oxidation and energy, as nitrate is reduced. In other plants that accumulate nitrate, as tomato, if the external source of nitrogen is chiefly nitrate, as it probably is in most tillable agricultural soils, there must continually be maintained

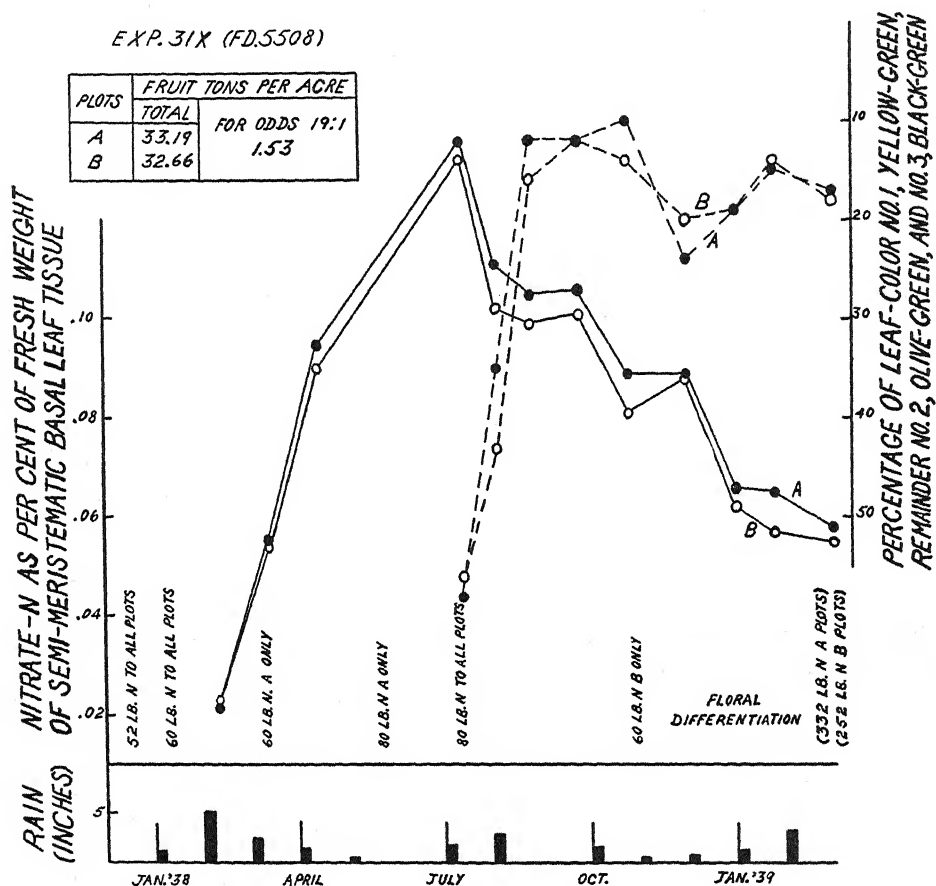


FIG. 14.—Prior to July, plants of plots A and B were filled to capacity with nitrate. Nitrate dropped after July, but in neither treatment became deficient in relation to carbohydrates, for no. 1 color after July was about 15 per cent or lower, indicative of low carbohydrate reserves. Accordingly, heavier nitrogen applications to A than to B were ineffective. Tables 1-6 give positive effects of nitrogen added to high-carbohydrate plants; tables 7-10 give additional examples of negative effects of nitrogen added to low-carbohydrate plants.

in the plant a fairly high concentration of nitrate to insure a synthesis from it of organic nitrogen sufficiently rapid to permit vigorous growth (18). Rapid growth and protein synthesis from nitrate practically cease in tomato, and apparently in pineapple, considerably before the nitrate reserves are completely exhausted.

The C plots, as shown in table 5, were not deficient in nitrate at any time during the experimental period. It does not seem likely, however, that they received an excess of nitrogen, for at the time the buds became evident they were slightly below the arbitrary point of 15 per cent no. 1 leaf color—at which pineapple plants are considered to be deficient in starch reserves. Certainly, prior to the initiation of experiment 29 F 1 in field 5510, there was no over-fertilization with nitrogen, as indicated in June, for instance, when routine field records for the proximate site of the experimental area showed that leaf nitrate was 0.088 per cent, with 38 per cent no. 1 leaf color. A nitrate value under these circumstances of 0.100 per cent

TABLE 6

EXPERIMENT 29 F 3A: FIELD 5403, PLANTED OCTOBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NI- TRO- GEN (LB.)	10/18/40		11/19/40	12/12/40		12/26/40		2/14/41	TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	NO. 1	NITRO- GEN (LB.)*	DIFFERENTIA- TION		NO ₃	NO. 1	BUDS NO. 1		TOTAL	PLUS OR MINUS D	RE- QUIRED FOR ODDS 10:1
					NO ₃	NO. 1							
A. . . .	340	.032	21	60	.040	19	.052	12	22	400	33.77	+2.28	1.07
B. . . .	340	.032	21	80	.039	23	.053	13	21	420	33.99	+2.50	1.07
C. . . .	340	.032	21	100	.041	21	.056	14	19	440	34.63	+3.14	1.07
D. . . .	340	.032	21	None	.028	31	.030	19	32	340	31.49	1.07

* Ammonium sulphate applied in lower leaf axils November 9, but there was not sufficient rain to dissolve and carry it to the roots until November 19.

would not have been too high (fig. 8), but that the C plots received practically ideal nitrogen nutrition after initiation of the experiment, and that this was correlated with marked, significant increases in yields, has been noted. Obviously, not less than about 445 pounds of nitrogen per acre was needed for maximum fruit production in field 5510 under the very favorable climatic conditions that prevailed. This is in contrast to results to be reported for the crop a year earlier in field 5508 (fig. 14). The two fields adjoin each other and yet, while about 445 pounds of nitrogen per acre was required for the 1940 crop in field 5510, only about 252 pounds of nitrogen was needed for the 1939 crop, a difference of more than 75 per cent in nitrogen requirements in successive years in essentially the same location.

The following data pertain to the responses of nitrate-deficient pineapple plants under conditions of comparatively low temperature. In some respects the results for field 5403, typical of other cool locations (table 6), seem aberrant in relation

to the responses of other species, since low temperature apparently is not conducive to carbohydrate accumulation in pineapple. It is well known for many types of plants that moderately low temperatures, although not favoring rapid carbon-dioxide assimilation, tend to conserve carbohydrates already contained, as well as those newly synthesized. This is probably owing mainly to a decrease in rate of respiration of sugars or their derivatives (18). In field 5403 the average 2-hourly air temperatures were—as usual—relatively low, being respectively about 67° , 65° , and 63° for December, January, and February of the experimental period at the nearest weather station, about a mile away. Actually the air temperatures at the site of the experiment were probably 1° – 2° lower, as judged from occasional temperature readings. The light rate for the same months at the nearest weather station was about 5000 foot candles. Considering their low nitrate content at the initiation of the experiment (table 6), however, the plants were not very high in carbohydrates, as indicated by only 21 per cent no. 1 leaf color.

Another notable feature is that at time of differentiation, although white root tips were abundant and soil moisture adequate, leaf nitrate had been raised from 0.032 to only 0.041 per cent by 100 pounds of nitrogen added about a month before. This figure is in contrast to 0.075–0.086 per cent at differentiation, which, when coupled with adequate carbohydrate reserves, has been shown to be essential for maximum production of fruit (tables 1–5). But that such high nitrate values, if practically attainable, in field 5403 would probably not have increased yields but would have been associated with extreme carbohydrate deficiency, is indicated by the low percentage of no. 1 color (12–14 per cent) in the nitrogen-supplied plants of plots A, B, and C. The plants displayed this leaf color on December 26, even though nitrate at that time was 0.056 per cent, which may be compared with a maximum possible value of about 0.085 per cent (fig. 8). There was seemingly a low rate of absorption of nitrate; on the other hand, with the considerable increases in yields associated with increased nitrate reserves (table 6), it does not seem likely that reduction of nitrate and new protein synthesis that occurs mainly in the leaves (24, 25) could have been seriously limited by low air temperature. What influences the low air temperatures concerned may have had upon carbon-dioxide exchange is problematical. It is significant that the plants of plots A, B, and C, although low in actual percentage of nitrate, for the most part contained enough, in relation to carbohydrates, as indicated by leaf-color values. Although the plants in field 5403 were on an apparently comparatively low plane of nitrate and CO_2 nutrition, the condition of balance noted was correlated with increases in yields of 2–3 tons of fruit per acre as compared with the plants of the D plots that exhibited a condition of imbalance owing to nitrate deficiency (table 6).

SOIL TEMPERATURE AND NITRATE ABSORPTION.—Because carbohydrate reserves were low and large increases in nitrate would therefore have been unde-

sirable, the apparently slow rate of absorption of nitrate by the plants in field 5403 probably did not influence yields unfavorably, although if absorption had occurred more freely, lesser amounts of fertilizer might well have been employed. But if opportunity for carbohydrate accumulation, in this or another field, should at any time be much greater, then any factor that materially repressed absorption of nitrate would be decidedly disadvantageous. There may be several reasons for this limited uptake of nitrate. While certain desirable data are not at hand, SCHROEDER (23) found that cucumber roots absorbed practically no water at 55° and that absorption was about twice as rapid at 70° as at 60° . Similar information for pineapple does not appear to be available, but WATANABE (30) has shown that until a temperature of about 68° – 70° is reached, pineapple root tips elongate very slowly (fig. 9). It does not seem unlikely that the same temperatures that limit growth of roots would also repress other activities, including absorption of nitrate. While continuous records of soil temperature were not obtained and momentary readings taken during the day at monthly intervals (at a depth of about 8 inches between the plants at the edge of the mulch paper) leave much to be desired, determinations show that the soil temperatures in field 5403 on December 12 and 26, respectively, in the area of the experiment, were 69° and 67° . These temperatures may be compared with determinations in field 5510. There nitrate was apparently freely absorbed (table 5), and on the most nearly corresponding dates the soil temperatures were 73.4° and 72.5° . At least, when they were read, the soil temperatures in field 5403 were just about at the point where there is a sharp break in the curve for rate of root growth (fig. 9). A slight increase or decrease in temperature would probably affect the rate of growth and possibly the absorption of nitrate.

As already pointed out, the percentage of white root tips was comparatively high in field 5403. This evidence is not, however, contradictory to WATANABE'S (30) results (fig. 9). A high percentage of meristematic or semi-meristematic white root tip tissue does not always indicate that the roots are increasing in volume or elongating rapidly. It is just as indicative of a slow rate of maturation and of differentiation of secondary elements, including lignified xylem vessels, fibers, and suberized cells. This situation was exhibited by peach and apple roots that remained largely undifferentiated for weeks at 55° and yet increased in length very slowly. At 75° , elongation of roots was rapid, but so also was differentiation, and at no time was there as great a proportion of white root tips as at lower temperatures (16).

CARBOHYDRATE DEFICIENCY AT TIME OF FLORAL DIFFERENTIATION.—The striking increases in yields which occurred when nitrate deficiency was corrected by applications of nitrogen during the period of differentiation and development of flower buds have been noted. The results presented in tables 7–10 show that—during the same stage of growth—increases in concentration of nitrate in the pineapple

plant, when coupled with carbohydrate deficiency (as arbitrarily indicated by less than 15 per cent no. 1 leaf color), typically did not increase yields significantly and were sometimes associated with apparent decrease in production of fruit. In field 5426 the plants of all the plots were approximately filled to capacity with nitrate by the end of September (table 7). The plants of the B plots contained nearly the maximum possible nitrate reserve (fig. 8) and were on the borderline of carbohydrate deficiency, as they displayed only 14 per cent no. 1 leaf color in September, even though up to that time they had been provided with less than 200 pounds of nitrogen per acre in contrast to over 300 for plots A and AX.

The B plots were fertilized, however, with the objective of obtaining the maximum possible yields on the site of the experimental area without employing a

TABLE 7

EXPERIMENT 31 X: FIELD 5426, PLANTED NOVEMBER, 1937. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NI- TRO- GEN (LB.)	9/23/38		9/25/38	10/25/38		10/29/38	12/1/38		12/29/38		1/25/39		3/1/39		TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. I	NITRO- GEN (LB.)*	NO ₃	No. I	NITRO- GEN (LB.)	NO ₃	No. I	DIFFER- ENTIA- TION	NO ₃	No. I	BUDS	TO- TAL	PLUS OR MI- NUS B		SIG- NIFI- CANCE		
A.....	332	.105	6	None	.090	20	None	.079	26	.048	34	.058	23	.058	19	332	30.3	-0.70	None
B.....	192	.096	14	None	.080	21	60	.084	20	.062	22	.060	17	.058	22	252	31.0	None
AX.....	352	.096	10	80	.084	18	None	.084	19	.057	24	.064	18	.061	19	432	30.6	-0.40	None

* Ammonium sulphate applied in lower leaf axils September 9, but there was not sufficient rain to dissolve and carry it to the roots until September 25.

luxury supply of nitrogen. Consequently the plants were not supplied with additional nitrogen until they had built up a higher carbohydrate reserve, as indicated by an increase from 14 to 21 per cent no. 1 leaf color at the end of October. Nitrogen was then applied sparingly, employing only a 60-pound application because carbohydrate reserves were not higher. The B plots were at least not outyielded by the others receiving up to 160 pounds more nitrogen per acre (table 7). That the application of 80 pounds of nitrogen to the AX plots in September would be futile might be anticipated, for the limiting factor was carbohydrates and clearly not nitrate. The plants were filled nearly to capacity with it, but were definitely deficient in starch, no. 1 leaf color being only 10 per cent. Subsequently the plants of the AX plots built up a slightly higher carbohydrate reserve, but not sufficient to require a nitrogen application, for nitrate reserves remained high in relation to carbohydrates.

The A plots present an interesting situation in that, although supplied with a luxury amount of nitrogen, the timing of the applications of the different increments of it was so poor that at about the time of differentiation still more nitrogen was needed, for the plants had decreased to 0.048 per cent nitrate, too low (tables 1-6) for 34 per cent no. 1 leaf color with which it was coupled. A light application of about 60 pounds of nitrogen to the A plots at about time of differentiation might therefore have increased yields slightly, provided the fertilizer became immediately available.

Field 5518, when nitrate deficiency was corrected (table 1), showed striking increases in yield, up to 3.74 tons per acre. These favorable responses followed the application of nitrogen to plants that exhibited 21 per cent no. 1 leaf color. It is a

TABLE 8

EXPERIMENT 31 D: FIELD 5518, PLANTED NOVEMBER, 1939. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NI- TRO- GEN (LB.)	9/24/40		10/21/40		10/23/40	12/11/40		12/27/40*		TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. 1	NO ₃	No. 1	NITRO- GEN (LB.)	NO ₃	No. 1	DIFFEREN- TIATION			TOTAL	PLUS OR MINUS B	SIGNIFI- CANCE
									NO ₃	No. 1				
B.....	340	.059	19	.073	18	None	.062	22	.068	20	340	32.43	None
D.....	340	.060	21	.072	21	80	.072	20	.085	13	420	32.99	+0.56	None

* Budding in late February, 1941.

coincidence that the plants described in table 8, likewise in field 5518, just prior to a nitrogen application, also displayed 21 per cent no. 1 color. In contrast, however, they gave no significant increases in yield from added nitrogen, even though leaf nitrate was ultimately greatly increased. The answer to these seemingly divergent responses is not apparent until the relative nitrate reserves are considered, not only for the respective dates just prior to nitrogen treatments but in each case at an earlier date of analysis. As shown in the first case (table 1), leaf nitrate decreased rapidly in one month from 0.090 to 0.063 per cent, the latter figure being in combination with 21 per cent no. 1 color. In the second instance, where no significant increases in yield followed fertilization (table 8), there had been just prior to the nitrogen application an increase in nitrate, in a little less than one month, from 0.060 to 0.072 per cent, with no. 1 color remaining constant at 21 per cent. Evidently the addition of nitrogen to plants displaying this color, indicative of a rather low starch reserve, would seldom be considered advisable with nitrate in-

creasing rapidly and at the concentrations recorded. Eighty pounds of nitrogen, added to the plants of the D plots, increased nitrate to approximately the maximum possible level at differentiation, and there was a gain in yield—not significant—of only 0.56 of a ton per acre. But the high nitrate value was associated with low concentration of carbohydrates, for the plants exhibited only 13 per cent no. 1 leaf color. Unquestionably carbohydrates, and not nitrate, were the limiting factor to greater tonnage.

This discussion is concerned with the condition of the plant during the last 3-4 months before buds appeared, not particularly with the agricultural practices employed, with prior fertilizer treatments, or with the fluctuating array of environmental influences characteristic only of the season and of a specific site. On the other hand, the responses of plants, quantitatively described, furnish informa-

TABLE 9

EXPERIMENT 31 C: FIELD 5415, PLANTED NOVEMBER, 1938. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRI- OR NI- TRO- GEN (LB.)	8/16/39		9/12/39		9/21/39		10/20/39		11/21/39		12/26/39		2/6/40		TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. 1	NO ₃	No. 1	NITRO- GEN (LB.)	NO ₃	No. 1	PRE-DIF- FERENTIA- TION		NO ₃	No. 1	NO ₃	No. 1	TO- TAL		PLUS OR MI- NUS D	SIG- NIFI- CANCE	
									NO ₃	No. 1									
B...	360	.096	15	.084	12	None	.067	17	.062	31	.050	24	.039	23	360	38.03	+0.15	None	
D...	340	.089	18	.072	14	80	.087	16	.068	28	.054	20	.043	16	420	37.88	None	

tion that is of general use in subsequent years even though a vast number of different environmental patterns and fertilizer treatments may lead to the production of plants essentially similar in content of nitrate and carbohydrates. Thus, in table 9 it is not implied that either of the plots had an efficient schedule of nitrogen applications prior to August, although the nitrogen nutrition of the plants in earlier months of growth is discussed elsewhere. The fact is simply emphasized that the plants of the D plots, even though decreasing in nitrate, were bordering on carbohydrate deficiency shortly before they received 80 pounds of nitrogen on September 21. That this application of nitrogen materially increased the nitrate content a month later is apparent from table 9. Nevertheless, this was not correlated with any increase in tonnage, but rather with an apparent but not statistically significant loss. Negative effects from nitrogen added to plants low in carbohydrates is a characteristic response that has occurred repeatedly. The plants of the D plots thus responded typically, for just before and a month after nitrogen was applied they displayed, respectively, only 14 and 16 per cent of no. 1 leaf color—indicative

of low carbohydrate reserves. Further, nitrogen added when plants are deficient in carbohydrates frequently appears to be a total loss, even though subsequently there may be accumulated a more liberal carbohydrate supply, as in the plants of the D plots just before differentiation. These plants at that time might have given increased yields if nitrate had then been higher in relation to the prevailing leaf color, 28 per cent no. 1. On October 24, however, 4.2 inches of rain fell, and during the month there was nearly 7 inches. This may have resulted in removal of nitrate, in whole or in part, from the soil in the vicinity of the root systems.

Much additional evidence might be given to support that already presented. In field 5320 the responses were sufficiently different from the foregoing reports to

TABLE 10

EXPERIMENT 29 F 2: FIELD 5320, PLANTED NOVEMBER, 1938. YIELDS, POUNDS PER ACRE OF APPLIED NITROGEN, PERCENTAGE OF LEAF NITRATE-N (NO_3), AND PERCENTAGE OF LEAF AREA EXHIBITING YELLOW-GREEN COLOR (NO. 1), WITH REMAINDER OF LEAF AREA NOS. 2 AND 3 COLORS

PLOT	PRIOR NI- TRO- GEN (LB.)	10/16/39		12/1/39		12/1/39	1/15/40		2/19/40		TO- TAL NI- TRO- GEN (LB.)	FRUIT (TONS PER ACRE)		
		NO ₃	No. 1	PRE-DIFFER- ENTIATION		NITRO- GEN (LB.)*	NO ₃	No. 1	BUDS			TOTAL	PLUS OR MINUS A	RE- QUIRED FOR ODDS 10:1
A.....	372	.045	30	.055	22	None	.060	14	.045	14	372	31.92
B.....	372	.045	30	.055	22	80	.074	3	.051	3	452	32.81	+0.89	0.83

* Ammonium sulphate applied in lower leaf axils on November 16, 1939, but there was not sufficient rain to dissolve and carry it to the roots until after December 1, 1939.

make brief mention of them worth while. Before differential nitrogen treatments were introduced (table 10), the plants exhibited 30 per cent no. 1 leaf color and only 0.045 per cent leaf nitrate-nitrogen. The maximum possible nitrate content for this stage of growth is about 0.100 per cent (fig. 8). Knowing nothing about the climatic conditions characteristic of field 5320, the conclusion might be reached that these plants were definitely deficient in nitrate in relation to carbohydrates, as 30 per cent no. 1 color is indicative of a very liberal reserve of starch. While such was presumably the case, the plant condition that prevailed would not necessarily indicate that substantial increases in yields would occur following increase in nitrate reserves. In the first place, it should be noted that—without any ammonium sulphate being added—leaf nitrate increased during November from 0.045 to 0.055 per cent, and no. 1 leaf color during the same period decreased from 30 to 22 per cent. Soil nitrate fluctuates materially (2) and may have been in part responsible for the rise in leaf nitrate. But even more critical is the fact that the

light rate in fall and winter is typically low in the area of field 5320. For example, during the experimental period for November–January, the light rate was almost exactly 3500 foot candles each month. These conditions are not favorable for abundant accumulation of carbohydrates, particularly when—with about 16,000 plants per acre—the lower leaves are exposed to but a small fraction of the total light intensity. Correlated with the low light rate, even the A plots (to which no nitrogen was added during the experimental period) were just on the arbitrary borderline of carbohydrate deficiency in January and February, for they displayed only 14 per cent no. 1 leaf color. On the other hand, the B plots, to which 80 pounds of nitrogen was added the first of December, increased in leaf nitrate over A, but this was correlated with a decrease in no. 1 leaf color to 3 per cent. Associated with these responses was an increase of less than 1 ton of fruit per acre, just within the limits of statistical significance.

The foregoing experiments, with many similar ones not here reported, furnish a fair picture of the high degree of precision that can be attained in the nitrogen nutrition of pineapple. With plant records taken in routine field analyses at monthly intervals, however, and with weather for the succeeding month uncertain, absolute precision in fertilization is unattainable. Thus, if sunshine and temperature for any particular location were much more favorable for carbohydrate accumulation than would be anticipated from weather reports in other years, increased nitrate reserves might well be associated with gains in yields that would not usually occur. On the other hand, if carbohydrate accumulation were limited by unusually adverse weather conditions, gains in fruit tonnage from increases in the nitrate level would be lacking, or much less than anticipated.

As will be shown presently, extreme precision in the timing of nitrogen applications is apparently unnecessary in the earlier stages of growth. The foregoing remarks apply particularly, therefore, to the last 3 months before budding. But if plant records are taken as described and employed as a guide to nitrogen requirements, a contemplated application of nitrogen may usually be depended upon to produce certain definite results, even though the weather may be somewhat atypical for the site, except where plants are on the borderline of nitrate or carbohydrate deficiency. And under either of these conditions, a not very great shift in weather might materially influence the results obtained.

This report of investigations on only fruitful classes of pineapple plants, all within the range of types that for convenience KRAUS and KRAYBILL (10) placed in a single class, is strongly supported by the studies which RUSSELL and BISHOP (22) have made of barley, wherein they likewise employed only fruitful plants growing under field conditions within a relatively narrow range of practical productivity. After 10 years of research, they state—referring to nitrate and carbohydrate assimilation—“These two factors produce two entirely different effects. Up to a

certain quantity, increases in the nitrate supply in the soil correspondingly increase the nitrate uptake by the plant, and this correspondingly increases its carbohydrate assimilation. The yield thus increases, but the composition of both plant and of grain hardly changes. . . . Further increases in soil nitrate supply beyond this stage have a different effect: they raise the nitrogen content of the plant as a whole and of the grain, but do not correspondingly increase the yields. There is no sharp point where increased yield ceases and increased nitrogen content begins; both effects overlap, but the change is well marked around a certain nitrogen content." Thus, work with another plant, produced under the climatic conditions prevailing in England, gave results which, in principle, are in entire accord with those of pineapple grown in the semitropical environment of Hawaii. Obviously these principles (10) are not new or peculiar to pineapple, but they are of interest in that they have been directly applicable in increasing the effective use of nitrogen in the nutrition of this plant over very considerable acreages.

NITRATE DEFICIENCY DURING EARLY VEGETATIVE STAGES AND SUBSEQUENT RECOVERY.—It is apparent that the period at which differentiation of flower buds takes place is a critical stage of growth. If at that time nitrate is deficient in relation to carbohydrates, as indicated by plant records, pronounced losses in yield of fruit may be prevented if the deficiency is promptly corrected by addition and absorption of nitrogen. It has also been shown that—even though the concentration of nitrate in the plant may be extremely low—added nitrogen, whether or not it raises the nitrate reserves, may be ineffective if applied to plants deficient in carbohydrates. In contrast, during the earlier stages of growth there is much more latitude. Even when carbohydrate reserves are abundant, as indicated by no. 1 leaf-color values materially higher than 15 per cent, there may be 3-4 months during which nitrate reserves decrease to much lower than the maximum values shown in figure 8, with relatively slight loss in yield of fruit (fig. 12). This is true provided the plants are not deficient in nitrate in relation to carbohydrates during the period of floral differentiation and bud development. A few examples, typical of results that have appeared repeatedly, are given here.

Figure 10 shows that plots B and F both received 340 pounds of nitrogen per acre, but that the plants of the F plots significantly outyielded those of the B plot by 1.47 tons of fruit per acre. This was owing to the fact that the B plots received a predetermined schedule of nitrogen treatment which, within the imposed limit of the 340 pounds, appeared no less desirable than some other that might have been formulated in advance. In contrast, as soon as the plants became established, those of the F plots were fertilized on the basis of the determinations made of leaf nitrate and leaf color, due account also being taken of the absorptive capacity of the root system, as indicated by the percentage of white root tips and the root anchorage. The first differential treatment between the two plots occurred

in March, when the B plots received only 60 pounds of nitrogen. Leaf-color values were not available at that time, because the leaves had not expanded sufficiently to permit taking such records. But with nitrate content increasing rapidly at that time (fig. 10), any immediate application of nitrogen was not essential. In May the B group was again supplied with nitrogen, but this time 100 pounds was employed while the F plots received only 60. Nitrogen was added to the F group because of the flattening off of the nitrate curve and the very high percentage of no. 1 leaf color (fig. 10). More than 60 pounds of nitrogen was not applied, however, because the plants were still very small and the percentage of no. 1 leaf color was decreasing rapidly. One month later the leaf-nitrate content of the B group was about 0.100 per cent, approximately the maximum value attainable (cf. fig. 8). The F plots were slightly lower, 0.090 per cent. In August the plants of the F plots had dropped to 0.060 per cent leaf nitrate and displayed 35 per cent no. 1 leaf color. Additional nitrogen was therefore necessary, and 100 pounds was applied. The B group received 80 pounds. Note the very sharp rise in nitrate and shift from 35 to 20 per cent no. 1 yellow-green color (only in the F plots) about a month after the fertilizer was applied. Note further that the trend of the nitrate and color curves in figure 10 was apparently affected very little, if at all, by the nitrogen added to the B plots. The strong positive response of the F plots to added nitrogen and the seemingly negative reaction of the B groups are characteristic, not only of pineapple but of many other plants. Whatever the reason, it seems consistently true that plants deficient in nitrate and high in carbohydrates—when given a liberal external supply of nitrate—absorb and reduce it much more vigorously per unit of plant weight than do plants with a less abundant carbohydrate reserve and a higher nitrate content (18). Thus, during late summer, the plants of the F plots were definitely low in nitrate in relation to carbohydrates, and—as the plant weight records showed—had been growing in volume relatively slowly. They were nevertheless actively storing up a carbohydrate reserve, as indicated by 35 per cent no. 1 color. The time spent in carbohydrate manufacture and therefore not available for vigorous protein synthesis—owing to lack of a sufficient concentration of nitrate in the plant from which freely to manufacture it—was seemingly of little or no disadvantage. When nitrate was again made available, the high carbohydrate plants of the F plots apparently absorbed and reduced it far more actively than they would have, had carbohydrates been materially lower. The B plots, on the other hand, could not efficiently use, and did not need, the nitrogen application at the time they received it; for in August, just before they were provided with 80 pounds, they were on the borderline of carbohydrate deficiency, displaying only about 15 per cent no. 1 leaf color.

Clearly, this nitrogen could later have been used by the plants to advantage if it had remained available in the soil until the B plots, just before floral differentia-

tion, increased in starch reserves to the point where they displayed about 30 per cent no. 1 leaf color. But the nitrogen seemingly was not then available, possibly owing to leaching by fairly heavy rains (fig. 10). The favorable yield given by the F plots is probably owing in large part to the fact that 80 pounds of nitrogen applied in November, as the plants exhibited about 30 per cent no. 1 leaf color, brought them into the critical period of floral differentiation with about the maximum nitrate content that could be carried without incurring carbohydrate deficiency. In February, a little before budding, the plants of the F plots displayed only about 15 per cent no. 1 leaf color.

While perhaps of only incidental interest, it is shown in figure 10 that the leaf-color and leaf-nitrate curves do not always have similar trends. Nitrate reduction accompanied by carbohydrate oxidation, new protein synthesis, and often accelerated respiration (18) is only one of several factors that can cause a shift in leaf color and in concentration of carbohydrate reserves. Cloudy weather or shading as the plants become larger and more crowded will often result in darker green color, even if not so extreme as to induce etiolation. Both nitrate determinations and color readings have contributed to increased efficient use of nitrogen in fertilization of pineapple. Neither alone is adequate. For instance, even though nitrate is decreasing rapidly in the F plots from December to February, the plants are becoming greener (fig. 10). On the contrary, during March and April, while the plants of the B plots were approximately filled to capacity with nitrate, they did not decrease significantly in percentage of no. 1 leaf color until the third month, and then continued to increase in greenness for the next 3 months—although nitrate decreased during the same period.

The application of nitrogen just prior to floral differentiation that gave the favorable results shown in figure 10 is no reason for assuming that a similar fertilizer treatment might be effective for the next planting in the same field, as already indicated in tables 7-11. Because climatic conditions vary from year to year, the schedule of nitrogen fertilization cannot be made inflexible, but must vary in accordance with them.

The results shown in figure 11 are essentially similar to those already described for field 5477 (fig. 10), and there is little point in a complete review of the experimental manipulations and plant responses in the relatively warm field 5530. In spite of differences in yields (about 8 tons of fruit per acre between the two locations), owing in large part to unlike environment, the general principles involved are essentially the same. Within the imposed limit of 340 pounds of nitrogen, materially greater yields were obtained in the F plots, which at the time of differentiation contained an adequate nitrate reserve in relation to carbohydrates, even though during the earlier stages of growth they were relatively low in nitrate as compared with the B plots.

As a rule there have been enough experimental plots in any given field so that the various situations presented in figures 10-14 have often been demonstrated in a single location. The principles demonstrated by these responses of the plants are characteristic of the plant itself and not peculiar to an experimental site. Thus, fields 5477 and 5530 both show that if for any reason the nitrogen supply must be limited, it can best be withheld during the earlier stages of growth, not during the period of floral differentiation and development (figs. 10, 11). Yet field 5477, because of comparatively high elevation, has an average 2-hourly temperature for 12 months that is about 4° F. cooler than that of field 5530, a difference that in the aggregate is great and associated with much reduced plant size and yields.

The results shown in figures 10 and 11, and in tables 1-7, demonstrate the importance of maintaining at the time of differentiation an adequate nitrate reserve in relation to carbohydrates. Figure 12 illustrates the responses of two plots, both of which at differentiation were nearly filled to capacity with nitrate for that stage of growth (fig. 8) and on the borderline of carbohydrate deficiency, as indicated by slightly less than 15 per cent no. 1 leaf color. Obviously the plants of both plots were amply supplied with nitrate reserves at that time. Earlier, however, the F plots, which received 80 pounds less nitrogen in April than the D plots, were—except for a brief period in June—definitely deficient in nitrate. This was indicated by the fact that leaf nitrate, in relation to carbohydrate reserves, was much below 0.100 per cent, the proximate maximum value (fig. 8), and the plants exhibited about 30 per cent no. 1 color during most of the summer. In contrast, the plants of the D plots contained approximately as much nitrate as could have been carried without incurring carbohydrate deficiency, for they displayed during most of the summer about 20 per cent no. 1 leaf color, indicative of only moderate carbohydrate reserves. Associated with ample nitrate reserves at time of differentiation and bud development but with a deficiency during earlier stages of growth, as already noted, the F plots were significantly outyielded by only 0.91 tons per acre. This difference does not correspond to the losses in tonnage that often occur when nitrate is deficient in relation to carbohydrates at time of differentiation (tables 1-6).

Figure 13 shows just the reverse situation of that presented in figure 12. The plants of the B plots (fig. 13), deficient in nitrate in relation to carbohydrates only at time of differentiation, were significantly outyielded by the D group, which were continually supplied with adequate nitrate reserves. In fact, both lots of plants probably received during the summer somewhat more nitrate than they could efficiently metabolize, as carbohydrate reserves were then low, no. 1 leaf color being less than 10 per cent. For maximum possible yields, the objective in the D plots, it is obviously essential to keep the plants adequately provided with nitrate in relation to carbohydrates. And the supply of the latter depends in large part

upon the opportunity for carbon-dioxide assimilation. Because this is determined largely by weather conditions that are variable, it follows that an inflexible schedule of nitrogen applications is undesirable. If it should be desirable to limit nitrogen for reasons of cost, it seems certain that this had better be done relatively early, not during the period of floral differentiation or the early stages of bud development.

Figure 14 illustrates another situation, wherein the plants of the A plots received a predetermined schedule of nitrogen fertilization. The B plots were fertilized, employing the usual plant records as guide to nitrogen requirements. For some weeks in the early summer leaf-color readings were omitted. Drought was a limiting factor, as shown by records (not here reported) of severe leaf-water deficiency. Moisture in the white basal semi-meristematic tissue of the leaves was not noticeably depleted, and analyses showed that up to July the plants of both plots were filled to capacity with nitrate. This was true even though up to that time the B plants had received 140 pounds less nitrogen per acre than the A. Following rain in July, with increased plant volume and increased greenness (decrease in percentage of no. 1 leaf color), there was a sharp decline in nitrate reserves. This decrease was undoubtedly owing in large part to utilization of contained nitrate in new protein synthesis at a rate that exceeded nitrate absorption. Accordingly, 80 pounds of nitrogen was applied to the B plots. Following the predetermined plan of fertilization, the A plots were also provided with the same amount. From then on the plants of both plots contained approximately as much nitrate as could be carried without incurring carbohydrate deficiency. During most of the remaining period no. 1 leaf color was only a little above 15 per cent (fig. 14). As might be anticipated, with essentially no nitrate deficiency at any time in either plot, the difference in yield in favor of A of only 0.53 tons of fruit per acre was not significant, though in the former case there was employed 322 and in the latter 252 pounds of nitrogen. In another group of plots, 432 pounds of nitrogen was applied, but the yield did not significantly exceed the B series with only 252 pounds applied.

The experiments upon which this report is based were conducted only in areas where potassium and phosphorus were not limiting factors. Comparable results usually are not obtainable in soils low in potassium or phosphorus, or both. The leaf content of these elements must not be permitted to become deficient. By employing the quantitative records described, the determination and regulation of the nitrogen nutrition of pineapple plants becomes a comparatively precise procedure. Not only has this method placed the nitrogen fertilization of fields on a higher plane of efficiency, but the necessity of distributing large-scale experimental plots over a plantation is not essential. By means of quantitative records an experiment may be initiated at any time, often only 2 months before buds appear, and the effect of withholding or applying nitrogen on yields thus determined. Critical

study of the plant records and associated climatic conditions will show the reasons for gains or losses in yield.

NITRATE AND LOW TEMPERATURE AS CORRELATED WITH FLORAL DIFFERENTIATION.—All the experimental plots described in the present report were in fields planted in the fall and early winter. A year later the plants entered the winter period of low temperatures as nitrate was decreasing (tables 1-10; figs. 10-14). In some sites the plots high in nitrate in relation to carbohydrates fruited 2-3 weeks later than others that contained less nitrate but more liberal carbohydrate reserves. In other locations all the plots differentiated flowers at essentially the same time. This was apparently associated with intimate relationship between nitrate reserves, a single night or several nights of sufficiently low temperature, and the appearance about 5 days later of the earliest stages of floral differentiation recognizable under the microscope.

Similar responses have been recorded by THOMPSON (28, 29), PLATENIUS (20), KNOTT (9), and MILLER (15), for celery, onion, spinach, and cauliflower. Low temperatures in the field are often closely correlated with differentiation of flower buds. In the case of cauliflower, ROBBINS (21) has reported that nitrate deficiency directly or indirectly resulted in premature flowering. In pineapple, as already noted, both temperature and nitrate reserves appear to play a part in influencing the time of floral differentiation. Whether or not the photoperiod is of significance is not known. The maximum difference in length of day in Hawaii is 2.6 hours. Under suitable nutritional conditions, pineapple plants may differentiate flower primordia under the longest or shortest days that prevail. MCKINNEY and SANDO (13) report that "Sexual reproduction in the spring wheats and in the winter wheats is not dependent upon a critical temperature or a critical photoperiod. . . . However, the time when sexual reproduction occurs is greatly influenced by the temperature and the photoperiod." MELCHERS (14) has concluded that certain biennial plants do not blossom unless their growing points have been subjected to low temperatures. It may well be questioned whether it is the growing point or the leaves that are most responsive to effects of low temperature on time of floral differentiation.

The results just cited, and those of ROBBINS (21), indicate that both low temperature and low nitrate supply may influence the time of floral differentiation. In the case of pineapple also, these factors may be coupled with initiation of floral primordia. Attempts were made to estimate² the date of floral differentiation in

² There being an interval of 2 months between floral differentiation and emergence of buds, knowledge of the date of differentiation of a field is of considerable practical value in large-scale agricultural operations, for it furnishes notice of the proximate time of harvest of fruit 2 months earlier than does the date of budding. Of course, dissection of plants to determine whether they show floral differentiation is in general impractical, as it involves destruction of plants and would often have to be continued for weeks before differentiation was finally observable.

field and experimental plots, taking into consideration both the percentage of leaf nitrate-nitrogen and the minimum temperatures. An empirical formula was employed, wherein the percentage of leaf nitrate-nitrogen was multiplied by the minimum temperature on any given night. In general, when this value multiplied by this minimum temperature resulted in an empirical differentiation index figure of about 3.00 or less, the first stages of differentiation recognizable under the microscope occurred about 5 days later. There were frequent exceptions, for differentiation sometimes occurred even though the index figure was materially higher than 3.00, particularly when there were two or three nights of moderately low temperature within a period of 7-10 days prior to differentiation. As yet, no entirely satisfactory method has been found for equating effects of several moderately cool nights occurring consecutively or that lie within any one of several intervals of time, as compared with one cool night, with respect to their influence on the differentiation of plants of known leaf-nitrate values.

Coupled with minimum temperature, the weighted leaf-color values alone, or in conjunction with nitrate content, did not furnish as precise a method of forecasting the time of differentiation as did nitrate content alone. Perhaps this may be related to the fact that the white basal tissue of the leaf employed for nitrate analysis is in close proximity to the meristematic stem tip from which floral primordia arise, or that the nitrate content of the distal end of the stem corresponds closely with that of the white basal tissue of the leaf analyzed. But it seems unlikely that the percentage of nitrate is necessarily correlated directly with floral differentiation, since ammonium may be completely substituted for nitrate as an inorganic nutrient and yet the plants be vigorous and fruitful (24, 25).

It is possible that changes in percentage of nitrate accompany quantitative or qualitative changes in the stem tip that induce the synthesis of hormones, compounds which tend toward floral differentiation in conjunction with low temperatures. Even so, it is certain that, at low levels of nitrate supply, differentiation takes place although temperatures are continuously relatively high.

Thus plants comparatively high in nitrate have been found to differentiate floral primordia following a single night of relatively low minimum temperature or several nights of moderately low minimum temperature. On the contrary, plants extremely low in nitrate, or entirely lacking it, often differentiate floral primordia in the hottest months of the year, as when absorption of nitrate is largely prevented by inadequate root systems. Accordingly, in experiments where the plants of different plots are radically different in nitrate reserves, a single night of sufficiently low temperature may be coupled with simultaneous differentiation in all plots. On the other hand, if minimum temperatures are relatively high, the plots with plants low in nitrate often fruit considerably in advance of others that have a higher content.

When fields are planted in the spring, fruiting may frequently be delayed several months by means of heavy fertilization with nitrogen, provided low soil temperatures, or lack of potassium, or other limiting factors do not prevent the free absorption and accumulation of a high nitrate reserve. On the other hand, greatly delayed fruiting typically does not occur in a field planted in the fall. When a field set out in the spring enters the coldest winter months, it is still relatively young, and if there is available a liberal external supply of nitrogen that may be readily absorbed, the plants may contain the highest possible percentage of nitrate, for they are at the stage of growth corresponding to the peak of the curve for leaf nitrate (fig. 8).

Accordingly, unless the spring-planted plants are low in nitrate, the lowest temperature of winter may apparently be without influence on the direct or indirect induction of floral differentiation. As the plants become older, there is correlated with additional months of active growth—but not necessarily with seasonal conditions—a decrease in nitrate that is practically unpreventable. Such a trend is indicated in figure 8. By this time the season of lowest temperature often has passed. And with seasonal temperatures not low enough to play a major role in induction of floral differentiation, directly or indirectly, low nitrate reserves foster early fruiting, while plants higher in nitrate in relation to carbohydrates fruit relatively late. In contrast, fields planted in the fall or early winter are at a stage of growth about a year later when the maximum possible concentration of nitrate is so low (fig. 8) that directly or indirectly a single night of relatively very low temperature, or several nights of moderately low temperature, may apparently induce simultaneous floral differentiation in plots which show variations in nitrate reserves, as indicated in the tables and graphs included in this paper.

As might be anticipated, however, there may be cases in which—because of drought or low air temperatures, or both—growth in the summer or early fall is practically suspended for a period of 2–3 months. Such a field, when it enters the winter season, may still be young in terms of months of growth and at the proximate point of highest possible nitrate content (fig. 8). In such instances, as is true for plantings made in spring, temperatures—even during the winter—are seemingly not low enough directly or indirectly to induce floral differentiation in plants so high in nitrate. Such differentiation may then be delayed, sometimes for nearly a year. If nitrate decreases rapidly enough so that it is low during the warmer months, the time of differentiation may be intimately associated with the prevailing nitrate and carbohydrate reserves in the plants. If nitrate does not decrease materially until the early months of the following winter, then the date of differentiation may approximately coincide with a single night of very low minimum temperature or follow shortly two or three nights in close succession of moderately low minimum temperatures.

Summary

1. Methods are given for obtaining in the field quantitative records of plant weight, number and dimensions of leaves, and percentage deficiency, if any, of stored water in the leaf of the pineapple plant.

2. The potential absorptive capacity of the root system is indicated by records of root anchorage in pounds and percentage of main roots and lateral rootlets displaying white non-suberized tips.

3. Only white, semi-meristematic tissue at the leaf base is analyzed for nitrate, potassium, and phosphorus. Regardless of age in months, prior to blossom-bud development, the leaf employed for analysis is always in precisely the same easily recognized stage of development (fig. 6).

4. After emergence of the determinate blossom cluster, no more analyses for nitrate are made, for the external nitrogen supply after budding is essentially without influence upon the development of the original plant and the fruit it may produce.

5. Until the specialized storage tissue of the leaf becomes practically depleted of water, the white basal tissue does not fluctuate materially in percentage of moisture.

6. Macrochemical analyses for starch, the major carbohydrate reserve expressed as percentage of either green or dry material, completely failed to indicate to what percentage of capacity the plants were supplied with starch. This was owing to the fact that relatively xeromorphic yellow-green plants contained such a high proportion of inert lignified elements that, even though essentially all non-senescent starch-storing parenchymatous tissue was filled to capacity with starch, expression as percentage of the fresh or dry material indicated relatively small amounts of starch. With respect to nitrate nutrition, such a plant is not considered to be deficient in carbohydrates, even though the absolute amount is low.

7. In contrast, black-green, soft, succulent plants containing only about 25 per cent of the starch which their tissues were potentially capable of storing actually had much more starch than the woody ones, whether this content was expressed on the per cubic centimeter basis or as percentage of fresh or dry material. With respect to nitrate nutrition, plants in this category are therefore considered deficient in carbohydrates even though the absolute amount of starch is higher than in the former case.

8. It was determined by means of microchemical observations that the degree of greenness of the leaves reflected what percentage of the total capacity of starch reserves the plant possessed. The greater the degree of yellow-green color of the plant, provided it did not approach senescence, the higher the relative starch content in the sense noted. By employing suitable standards of color (fig. 7), the relative greenness, quantitatively recorded, supplied a sufficiently precise index of

carbohydrate content. Without this index, studies on nitrogen nutrition of pineapple, given in part in this paper, would have been of less significance.

9. It is essential to maintain an adequate reserve of nitrate in the plant. If the concentration of nitrate is relatively low, even though measurable amounts are present, its reduction is not so efficiently or freely effected as when it is higher. Sufficient carbohydrates must be available for oxidation as nitrate is reduced.

10. Plants which had been allowed to accumulate a high carbohydrate reserve and to become temporarily deficient in nitrate, when again supplied with nitrate, apparently absorbed and reduced it much more vigorously than other plants continuously higher in nitrate and lower in carbohydrates. Probably for this reason, provided the time until budding was adequate for plants to recover in content of nitrate and synthesize new protein, there was relatively little loss in yield of fruit, as compared with other plants that were at no time deficient in nitrate relative to carbohydrates.

11. If at any time prior to emergence of flower buds, nitrate reserves were deficient in relation to carbohydrates for any considerable length of time, yields of fruit were less than when the deficiency had been promptly corrected.

12. Even when the concentration of nitrate in the plants was extremely low and carbohydrates were also deficient, the addition of nitrogen—whether or not it increased the nitrate reserve—did not result in materially increased yields. In some cases there was an apparent decrease in yield.

13. There was some evidence to indicate that soil temperatures of 68° F. or lower result in limitation of absorption of nitrate by the roots.

14. During seasons when minimum temperatures are not low, plants deficient in nitrate and high in carbohydrates may fruit months earlier than others that contain a more liberal nitrate reserve but a lower concentration of carbohydrates. Following a single night of relatively low temperature or several nights when the temperature was not so low, however, plants comparatively high in nitrate—as well as others that are lower—may apparently differentiate flower primordia simultaneously.

The writer wishes to express his sincere appreciation of the assistance and enthusiastic co-operation of W. Y. WHANG, who supervised the workers who obtained field and laboratory records. He is likewise greatly indebted to W. A. WENDT and N. E. NEWPORT, who were responsible for agricultural operations and harvesting fruit, to LAWRENCE QUINN for careful statistical studies, and to members of the Pineapple Research Institute, particularly JOHN FO, CARL FARDEN, and H. E. CLARK.

LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official and tentative methods of analysis. 5th ed. Washington, D.C. 1940.
2. BATHAM, H. N., and NIGAM, L. S., Periodicity of the nitrate content of soils. *Soil Sci.* 29: 181-190. 1930.
3. BOYNTON, D., REUTHER, W., and CAIN, J. C., Leaf analysis and apparent response to potassium in some prune and apple orchards: Preliminary report. *Proc. Amer. Soc. Hort. Sci.* 38:17-20. 1941.
4. DAVIDSON, O. W., and BLAKE, M. A., Nutrient deficiency and nutrient balance with the peach. *Proc. Amer. Soc. Hort. Sci.* 35:339-346. 1937.
5. FARDEN, C. A., A method for obtaining and computing sunshine values from temperature data. Unpublished results, Pineapple Producers Co-operative Association, Honolulu, Hawaii.
6. HAYWARD, H. E., and LONG, E. M., The anatomy and roots of Valencia orange. *U.S. Dept. Agr. Tech. Bull.* 786. 1941.
7. HAYWARD, H. E., and BLAIR, W. M., Some responses of Valencia orange seedlings to varying concentrations of chloride and hydrogen ions. *Amer. Jour. Bot.* In press.
8. HOLMAN, RICHARD, On solarization of leaves. *Univ. Calif. Publ. Bot.* 16:139-151. 1930.
9. KNOTT, J. E., The effect of temperature on the photoperiodic response of spinach. *Cornell Univ. Agr. Exp. Sta. Memoir* 218. 1939.
10. KRAUS, E. J., and KRAYBILL, H. R., Vegetation and reproduction with special reference to the tomato. *Oregon Agr. Exp. Sta. Bull.* 149. 1918.
11. LAGATU, H., and MAUME, L., Le diagnostic foliare de la pomme de terre. *Ann. Ecole Nat. Agr. Montpellier. Premier memoire* 20:219-281. 1930.
12. MAGNESS, J. R., DEGMAN, E. S., and FURR, J. R., Soil moisture and irrigation investigations in eastern apple orchards. *U.S.D.A. Tech. Bull.* 491. 1935.
13. MCKINNEY, H. H., and SANDO, W. J., Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. *Jour. Agr. Res.* 51:621-641. 1935.
14. MELCHERS, G., Die Blühormone. *Ber. Deutsch. Bot. Ges.* 57:29-48. 1939.
15. MILLER, J. C., A study of some factors affecting seed-stalk development in cabbage. *Cornell Univ. Agr. Exp. Sta. Bull.* 488. 1929.
16. NIGHTINGALE, G. T., Effects of temperature on the growth, anatomy, and metabolism of apple and peach roots. *BOT. GAZ.* 96:581-639. 1935.
17. ———, The biochemistry of the nitrogenous constituents of the green plants. *Ann. Rev. Biochem.* 5:513-524. 1936.
18. ———, The nitrogen nutrition of green plants. *Bot. Rev.* 3:85-174. 1937.
19. NIGHTINGALE, G. T., and FARNHAM, R. B., Effects of nutrient concentration on anatomy, metabolism, and bud abscission of sweet pea. *BOT. GAZ.* 97:477-517. 1936.
20. PLATENIUS, H., Carbohydrate and nitrogen metabolism in the celery plant as related to premature seeding. *Cornell Univ. Agr. Exp. Sta. Mem.* 140. 1931.
21. ROBBINS, W. R., NIGHTINGALE, G. T., and SCHERMERHORN, L. G., Premature heading of cauliflower as associated with the chemical composition of the plant. *New Jersey Agr. Exp. Sta. Bull.* 509. 1931.
22. RUSSELL, E. J., and BISHOP, L. R., Investigations on barley. Report on the ten years of experiments under the Institute of Brewing Research scheme; 1922-1931. *Jour. Inst. Brewing* 39:287-421. 1933.

23. SCHROEDER, R. A., The effect of root temperature upon the absorption of water by the cucumber. Missouri Univ. Agr. Exp. Sta. Res. Bull. 309.
24. SIDERIS, C. P., KRAUSS, B. H., and YOUNG, H. Y., Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. 13:489-527. 1938.
25. SIDERIS, C. P., KRAUSS, B. H., and YOUNG, H. Y., Distribution of different nitrogen fractions, sugars, and other substances in various sections of the pineapple plant grown in soil cultures and receiving either ammonium or nitrate salts. Plant Physiol. 14:227-254. 1939.
26. SZENT-GYORGYI, A. V., On oxidation, fermentation, vitamins, health, and disease. Williams and Wilkins Co., Baltimore. 1939.
27. THOMAS, W., and MACK, W. B., Foliar diagnosis: The influence of the soil on the action of fertilizers. Plant Physiol. 14:75-92. 1939.
28. THOMPSON, H. C., Premature seeding of celery. Cornell Univ. Agr. Exp. Sta. Bull. 480. 1929.
29. THOMPSON, H. C., and SMITH, O., Seedstalk and bulb development in the onion (*Allium cepa* L.). Cornell Univ. Agr. Exp. Sta. Bull. 708. 1938.
30. WATANABE, SHOICHI, Effect of temperatures upon the root development of pineapples. I. The maximum, minimum, and optimum temperatures for the elongation of main roots. Communications, Hort. Inst. Taihoku Imperial Univ. no. 24. 1932. (Japanese translation by K. SAKIMURA.)
31. WATSON, R., and PETRIE, A. H. K., Physiological ontogeny in the tobacco plant. 4. The drift in nitrogen content of the parts in relation to phosphorus supply and topping, with an analysis of the determination of ontogenetic changes. Australian Jour. Exp. Biol. Med. Sci. 18:313-340. 1940.
32. WAUGH, J. G., and CULLINAN, F. P., The nitrogen, phosphorus, and potassium content of peach leaves as influenced by soil treatments. Proc. Amer. Soc. Hort. Sci. 38:13-16. 1941.
33. WILLIAMS, R. F., Physiological ontogeny in plants and its relation to nutrition. 4. The effect of phosphorus supply on the total-, protein-, and soluble-nitrogen contents, and water content of the leaves and other plant parts. Australian Jour. Exp. Biol. Med. Sci. 16:65-83. 1938.



MEIOTIC COILING IN TRADESCANTIA

C. P. SWANSON

(WITH NINETEEN FIGURES)

Introduction

Despite considerable research on chromosome coiling (see 7, 9, 16 for reviews), apparently no strictly quantitative attack on the problem has as yet been attempted, although this method offers one of the better avenues of approach to a more complete understanding of the coiling mechanism. It seemed worth while, therefore, to investigate the effects of a wide range of temperatures on meiotic coiling from a quantitative as well as from a qualitative point of view. This paper deals largely with the data thus obtained, together with data on the origin and development of the major coils in meiotic prophase stages. Some quantitative data on the genotypic control of major coiling are also included.

MATERIAL AND METHODS.—The genus *Tradescantia* has been used exclusively in this study. Two clonal lines were available, one of *T. paludosa* Anders. & Woodson and the other of *T. canaliculata* Raf. The meiotic chromosomes of the latter were statistically longer than those of *T. paludosa* and consequently were more easily analyzed, so that this line was more extensively used than the other. The results from both clonal lines, however, were closely parallel in all respects.

The plants were grown in pots during the fall and winter of 1940-41 in the greenhouses of the Biological Laboratories, Cambridge, Massachusetts. The average temperature of the houses was approximately 22° C. during the day but fluctuated so much during the time of the experiment that data from buds taken directly from the greenhouses were of little critical value. A considerable amount of data was gathered from such buds, however, and the average of these figures was used as an arbitrary control. For temperatures above greenhouse range, heat chambers were utilized, while constant-temperature coldrooms permitted the use of temperatures below 22° C. Both whole plants and cut inflorescences provided the cytological material, and since the results from both sources showed no significant deviations, the data were considered together.

TERMINOLOGY.—During meiotic prophase the number of major coils was greater than at meiotic metaphase. The prophase coiling cycle in meiosis (and mitosis as well) in *Tradescantia* is characterized by a progressive reduction in the number of coils. It is therefore necessary to conceive of the chromosome during the coiling cycle—not as a system becoming progressively coiled or spirialized as it passes from prophase to metaphase—but as one becoming progressively uncoiled or de-

spiralized. It has also been necessary, as a result of these observations, to separate the prophase coiling cycle into two stages: (1) the spiralization phase, at which time a relatively straight chromonema is transformed into a regularly coiled system; and (2) the despiralization phase, during which the numerous coils formed during the spiralization phase are reduced by an uncoiling which must take place at the distal ends of the chromosome arms, and which undoubtedly is accompanied by a rotation of ends within the matrix.

Observations

GENOTYPIC CONTROL OF MAJOR COILING

Numerous instances of an intraspecific genotypic control of meiotic chromosome length have been reported since LESLEY and FROST'S (11) classical case in *Matthiola incana* (2). In this plant the long chromosome form was recessive to the normal short chromosome form. A similar instance of altered chromosome length acting as a single Mendelian recessive has been described by UPCOTT (24) in *Lathyrus odoratus*, although the reverse was true in this case, the normal form possessing long chromosomes and the mutant male-sterile for the short chromosomes. LAMM (10) found a wide range of meiotic contraction in various segregates of inbred stocks of rye. In *Macronemurus*, a Neuropteran insect, NAVILLE and DE BEAUMONT (14) showed the chromosomes in the male to be more contracted than in the female.

In *Tradescantia*, different chromosome lengths were observed in the two clonal lines utilized in this study. Under average greenhouse conditions, *T. canaliculata* showed 8.1 major coils per chromosome (fig. 6), and *T. paludosa* an average of 6.08 (fig. 7), the length of the metaphase chromosomes being correspondingly long or short. Only metaphase chromosomes were used in determining counts, since it was found that the number of coils is progressively reduced during anaphase (fig. 8). The number of coils for *T. canaliculata* is much above the figures given by other investigators for diploid species of *Tradescantia* (15, 17).

The two clonal lines were crossed, and F_1 and F_2 populations were grown, although the number of plants in each generation was rather small. Six F_1 plants gave varying counts in the number of major coils at metaphase, the figures ranging from 6.56 to 7.64. In this respect they were intermediate between the two parental averages, but there was a noticeable trend toward the longer chromosome form of *T. canaliculata*. Little significance can be attached to this because of the few plants used and because of possible temperature fluctuations. Eighteen F_2 plants, obtained by crossing the plants with 6.56 and 7.64 coils, respectively, showed an extremely wide range of coiling, the lowest being 4.4 and the highest 7.64 coils per chromosome. Eight out of the eighteen plants showed a lower number of coils than did *T. paludosa*. The data are inadequate for drawing definite conclusions as

to the exact genotypic control, but it is at least apparent that—unlike the instances cited in *Matthiola* and *Lathyrus*—the situation is more complex than a single gene difference, and that both parental plants are heterozygous for genes influencing the degree of spiralization.

From numerous observations on other species of *Tradescantia*, *T. paludosa* appears more nearly to represent the average degree of spiralization for the genus, and apparently the increased length of the *T. canaliculata* chromosomes is due to a difference in the timing relationships during prophase. This difference may be expressed as: (1) normal spindle formation with a slower rate of despiralization, or (2) normal despiralization with a precocious spindle formation. Either would lead to increased chromosome length at metaphase. These observations in no way imply that spindle formation exerts influence on the process of despiralization during prophase. However, since only metaphase configurations were used in this study, since metaphase is a relatively brief yet conspicuous stage in meiosis, and since the onset of metaphase is governed by the development of the spindle, it seems justifiable to view the rate of despiralization and the time of spindle formation as the two most important factors which determine the degree of coiling to be found in any particular cell at metaphase.

To distinguish between these two factors would require a more intimate knowledge of prophase development in *Tradescantia* than is available at present. URCOTT (24) has been able to ascertain with considerable exactitude the behavior of the normal and the short-chromosome mutant in *Lathyrus*, and she finds that the timing of prophase, as judged from anther size, is such that in the mutant the time period for despiralization is so lengthened as to permit the chromosomes to pass the stage of normal contraction and reach an abnormal contracted state. No detectable difference in anther size could be found between the two species used in this study. When comparing the preceding examples with *Tradescantia*, it is, of course, assumed that the difference in length of the meiotic metaphase chromosomes is directly related to differences in the degree of major coiling, although it is also possible that the chromonema length is under genotypic control (26).

ENVIRONMENTAL CONTROL OF MEIOTIC COILING

The ease with which the synchronization of meiotic phenomena may be disturbed is indicative of the delicate balance of the entire mechanism. Of the many environmental agents utilized for experimental purposes (18), none—with the possible exception of colchicine—shows any specificity of action, but all express their effect as a general upset in both cell and nuclear division. Recently GILES (5) has pointed out that this may be because their ultimate effect is on the hydration mechanism of the cell, which in turn is intimately associated with cytokinesis, spindle formation, and coiling (20, 9). Any agent that could alter prophase timing

relationships would show its effect through the coiling system, but in selecting an agent for a quantitative study of the environmental control of coiling, one necessary criterion was that, when varied, it would provide detectable differences of a uniform and continuous nature without totally disrupting the other cell processes. The use of temperature satisfied this requirement. Temperature also has the advantage of being readily controlled, as well as providing a wide and continuous range of conditions within which the plant can function properly.

With the exception of studies on chiasma frequencies (22, 25), most cytological investigations of thermal treatment of meiotic cells have been qualitative (18), with descriptions of the irregularities observed throughout the various stages of pollen development. Comparatively little has been done quantitatively, perhaps largely because the changes arising from such treatment do not readily lend themselves to quantitative analysis. The coiling mechanism, however, provides a phenomenon which can yield an effective measure of the differences induced under controlled conditions, for as NEBEL (15) has already pointed out, and as this study shows, the number of coils per chromosome under constant conditions is a relatively stable and constant character. MATSUURA (12, 13), HUSKINS and WILSON (6), and WILSON and HUSKINS (26), using *Trillium*, were the first to present data showing the effects of thermal treatment on the number of coils. MATSUURA found that treatment at 16° C. (18° C. being considered normal) for 2 weeks reduced the number of major coils to 64 per cent that of normal, whereas 2 months at the same temperature reduced the number to 44 per cent. WILSON and HUSKINS showed that low temperatures lessened the number of coils as well as shortened the length of the chromonemata, while temperatures of 20° C. frequently prevented coiling. This latter effect is similar to that found in *Fritillaria* by BARBER (1), in that prolonged treatment (2 days at 30° C.) brought about complete suppression of spiraling in some cells, while abbreviated heat shocks had no visible effect. STRAUB (22) found a mitosis-like type of coiling at high temperatures and greater contraction at subnormal temperatures. In root-tip cells of *Allium*, DARLINGTON and LA COUR (3) observed that the chromosomes were markedly shortened when subjected to 0° C. for 5 days, although they also stated that at this "marginal temperature" the degree of coiling was variable.

There seems to be some consistency, then, as to the effects of temperature on the visible expression of coiling. Low temperatures reduce the number of major coils as well as shorten both chromosome and chromonema lengths, while high temperatures may completely inhibit the development of coils or cause the meiotic chromosome to assume a mitosis-like form. Since the qualitative effects seem well established, the main problem of the present research was therefore a quantitative one, with observations to be made over a wider and more continuous range of temperatures. The effects of thermal shock were disregarded in this study, and to al-

low for this, the plants were grown for at least 2 weeks at each temperature before any observations were made. Cold-treated plants were allowed longer periods of time to allow for the slowing down of the meiotic process which normally occupies a period of several days, although the exact meiotic timing relationships are uncertain because of the inadequacy of prophase configurations. This would permit examination of cells which had come up from a premeiotic state and had passed through the complete meiotic prophase cycle at the desired temperature.

No attempt has been made to measure chromonema lengths in the manner that HUSKINS and his co-workers have done in *Trillium* (6, 21, 26). In fact, in a double-coiled system of major and minor coils, such as is found in *Tradescantia*, measurements of chromonema lengths are virtually impossible since the minor coils are

TABLE 1

TEMPERATURE (° C.)	T. CANALICULATA		T. PALUDOSA	
	NO. OF CHROMOSOMES	COILS PER CHROMOSOME	NO. OF CHROMOSOMES	COILS PER CHROMOSOME
8.....	25	5.92	100	4.02
15.....	50	7.36	100	5.17
22.....	150	8.10	125	6.08
24.....	25	8.52
27.....	25	8.92	25	6.88
29.....	18	7.11	10	6.60
33.....	50	7.64	50	5.96
34.....	75	6.04
36.....	75	5.17	75	6.12
40.....	40	4.15	100	5.10
42.....	195	4.83

rarely so clearly differentiated that the diameter, pitch, and number of coils can be readily ascertained. The data and conclusions presented here are therefore derived from observations of major coils only, and—as a rule—where the major coils are reduced in number, the chromosome length is similarly reduced. Also, as the major coils are numerically reduced, the diameter of the major gyre is correspondingly increased, and it is probable that the proximal gyres achieve a greater diameter at the expense of the distal ones, which become progressively uncoiled.

The data from both clonal lines of *Tradescantia* are given in table 1 and presented graphically in figure 1. In compiling the data, the number of coils was always interpreted in terms of whole numbers, no attempt being made to determine half coils. In other words, the number of coils in a single chromosome might be 6 or 7 but not 6.5; this leads to some error, but it would be unavoidable in any case, because of the difficulty of following the coils.

From figure 1, it is evident that an essentially unimodal curve holds for both

species when the number of major coils per chromosome is plotted as a function of temperature. The peak in both instances falls at, or around, 27°C . Figure 9 illustrates a typical cell of *T. canaliculata* at 27°C ., showing the large number of coils and the slenderness of the chromosomes; figure 10 shows a similar cell at 33°C ., the coil number being somewhat below that normal; figure 11 illustrates the reduced length and coil number at 8°C . In this respect the data do not bear out completely previous observations, for here both extremes of temperature lead to greater despiralization and hence to greater chromosome contraction.

No immediate explanation is at hand as to why the peaks of the curves should fall at 27°C . The answer probably lies in the relative effects of different thermal

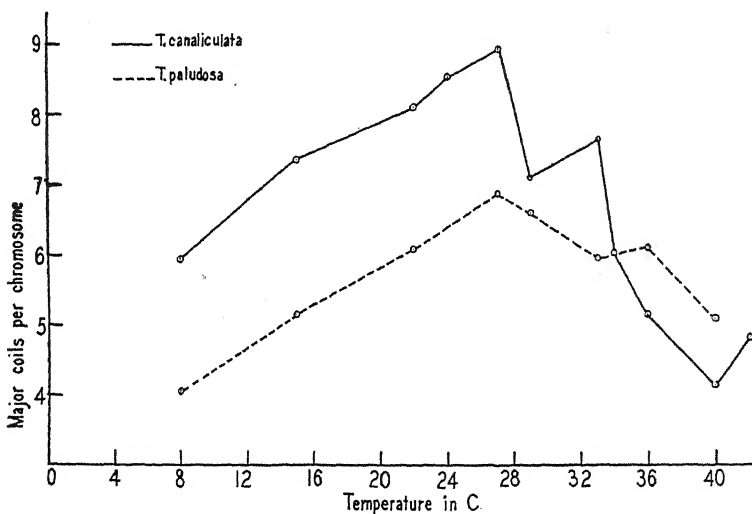


FIG. 1.—Graph illustrating effect of temperature on major coiling

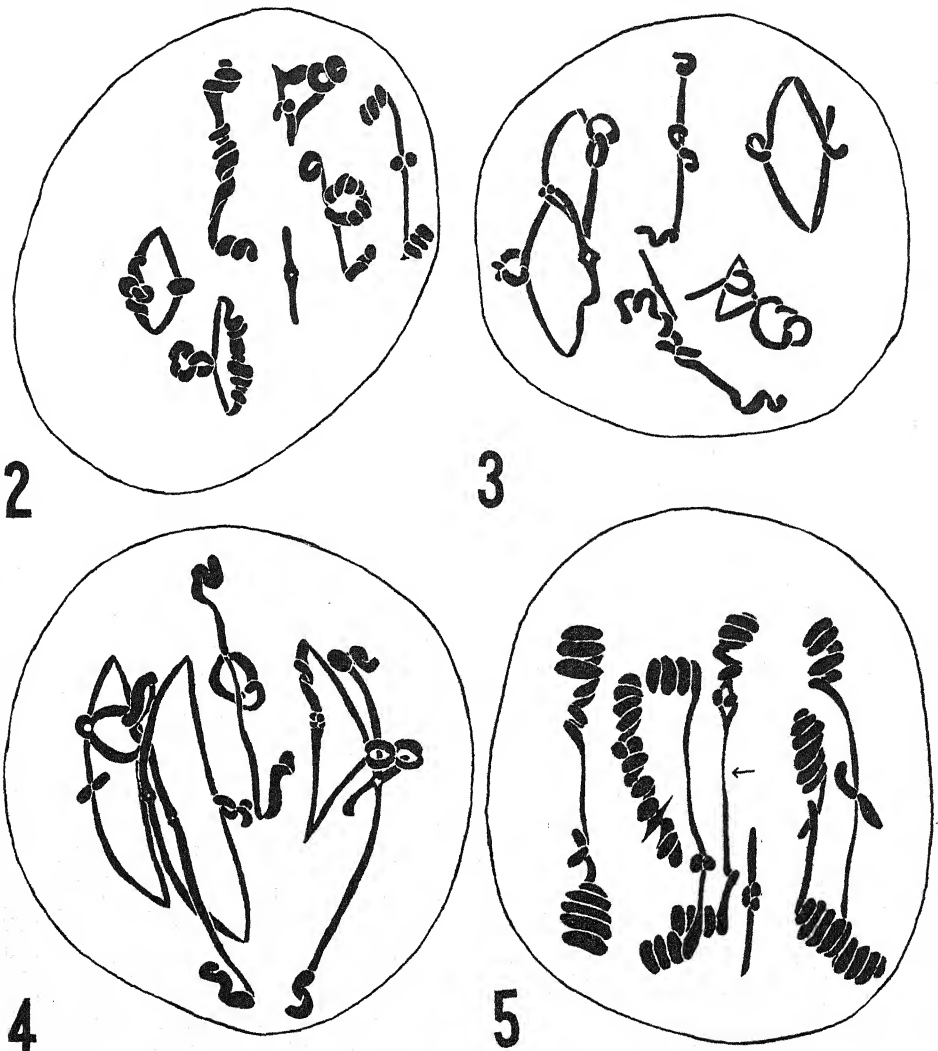
conditions on both the despiralization mechanism and the time of formation of the spindle, but as yet comparatively little is known of the chemical and physical nature of these processes, so that any hypothesis at this time would be suggestive only. In the light of other information, however, it seems likely that low temperatures delay the appearance of the spindle in some manner, thus permitting the coils to go beyond their normal stage of development, and giving in this way a shortened chromosome. At high temperatures, where the effect is more variable (fig. 1), the influence is an accelerating one but is more pronounced on the coiling system than on the spindle, with the result that the rate of despiralization is more rapid relative to spindle formation, thus permitting greater contraction—not because of a greater time interval but because of an increased despiralization tempo.

Variability in the degree of despiralization is greater at high temperatures. This

process must itself be, or is the result of, a chemical process, possibly due to matrix contraction or accumulation of nucleic acid around a protein core; but because all the coiling considered in this study was from metaphase configurations, and hence the expression of two inseparable variables (the coiling mechanism and spindle formation), it was impossible to obtain a temperature coefficient for chromosome contraction. Two slides of *T. canaliculata*, from inflorescences kept at 3.5° C., gave an average of 7.07 coils per chromosome, indicating a possible rise at extremely low temperatures; but since the inflorescences were at this temperature for only a week, and since circumstances prevented a checking of the data, the significance of this figure is questionable, and it was not plotted on the curve. A rise was also indicated in *T. canaliculata* at 42° C., but since temperatures above this killed the meiotic cells, no further data were obtainable. A lower chiasma frequency and a higher univalent frequency were usually found in cells which showed a low number of coils at both temperature extremes. The correlation was so striking as to indicate the probability that much of the movement of chiasmata, and possibly chiasma loss, is directly the result of the coiling mechanism (23). This, however, will be discussed at length in a later paper.

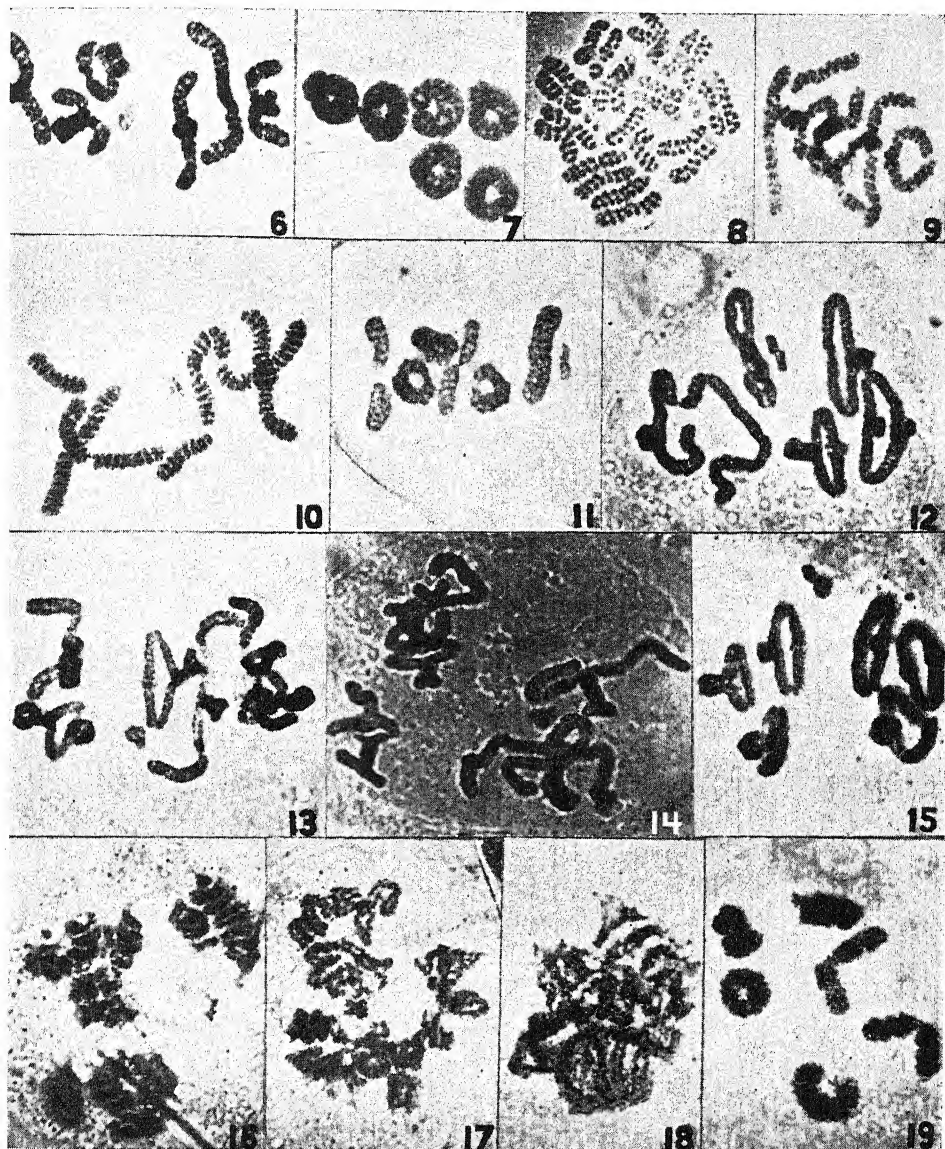
With the exception of univalents, the meiotic divisions throughout most of this thermal range were regular and constant, once the plants had recovered from their transfer from greenhouse temperatures. There was no noticeable upset of nuclear and cytoplasmic synchronization such as is characteristic of thermal shocks (18). At temperatures of 40° C. and above, however, striking differences were found in preparations from some buds in inflorescences that had otherwise almost normal material. The figures in table 1 and the points on the curves of figure 1, obtained at 40°–42° C., were included because they were judged to possess a normal, though somewhat increased, state of chromosome despiralization; but other slides at these temperatures were so anomalous as to be excluded from any such consideration. The behavior of these striking cells is to be seen in figures 2–5 and 12–19. All variations in the degree of despiralization were to be found, sometimes in a single anther, the number of major coils varying from thirty-two to as low as two per chromosome. The long chromosomes (figs. 3, 4, 12–15), designated 1-chromosomes from here on, appeared mitosis-like, except that they were paired as bivalents, whereas others appeared uncoiled to such an extent that the major coils were almost eliminated. This may be seen in figure 17, where the upper bivalent has the left-hand homologue spiraled into about one and one-half large coils while the right-hand homologue has lost its major coils. This figure also shows clearly the sister half-chromatids, which can separate laterally without entanglement. In some of the more sharply stained half-chromatids there is a suggestion of a third split (long arrows), giving an eight-parted chromosome possessing eight quarter-chromatids; but more observations are needed to make certain of this. The short

chromosomes (s-chromosomes) frequently show the minor coils, as might be expected under such conditions of extreme despiralization (fig. 16). Apparently in



FIGS. 2-5.—*T. canaliculata*, 40° C. Fig. 2, loose and irregular heat-induced coils. Fig. 3, early anaphase; lack of despiralization brought about by heat; lowermost bivalent failed to congress properly. Fig. 4, anaphase; marked lack of despiralization. Fig. 5, anaphase; considerable despiralization.

Tradescantia the minor coils follow a development parallel to that of the major, in that they reduce in number and increase in diameter as prophase progresses. This conclusion is based largely on inference rather than on direct observation, for the



FIGS. 6-19.—Fig. 6, *T. canaliculata*, normal metaphase. Fig. 7, *T. paludosa*, normal metaphase. Fig. 8, *T. canaliculata*, normal anaphase. Fig. 9, same, 27° C. Fig. 10, same, 33° C. Fig. 11, same, 8° C. Figs. 12-14, same, 40° C. Note lack of despiralization at high temperatures. Fig. 15, *T. paludosa*, 40° C. Fig. 16, *T. canaliculata*, 40° C.; arrows point to minor coils; marked despiralization. Fig. 17, same, 40° C.; short arrows point to sister half-chromatids; long arrows point to regions where further division has produced sister quarter-chromatids. Fig. 18, same, 40° C.; early diplotene; incipient major coils appear as dotlike chromomeres on parallel chromatids. Fig. 19, same, 40° C.; diakinesis; marked despiralization. X1000.

minor coils are difficult to follow in ontogeny; but in cells where the minor coils are greater in gyre diameter and fewer in number than in cells less advanced in prophase, it seems reasonable to assume that the minor coil development parallels that of the major coils, and that the fewer larger minor coils were derived from the more numerous smaller coils by a process of despiralization. This is not in agreement with COLEMAN and HILLARY'S (4) work in *Trillium*, for they state that the minor spiral decreases in diameter to give additional length to the chromonema.

Occasionally, after heat treatment, cells were found in various prophase stages not normally well differentiated in *Tradescantia*. Figure 18 is, so far as can be determined, an early diplotene configuration, with the chromatids visibly separated and the major coils at an incipient stage. They appeared to have been "thrown in," rather regularly, on the previously uncoiled chromonemata. Figure 19 shows the widely distributed chromosomes at diakinesis. This stage appeared frequently at 40° C., and although the bivalents possessed the fuzzy appearance characteristic of diakinesis, some were sufficiently well differentiated so that the minor coils could be detected. Later it was found that the same prophase stages, as well as the coiling anomalies, could be induced by transferring cut inflorescences from the greenhouse to an oven held at 40° C. for 24 hours, as well as by growing the entire plant at 40° C. for 2 weeks.

Even though at 40° C. the coiling system seemed at first to show all degrees of despiralization, analysis indicated that the cells could be separated into two distinct groups with very little overlapping: (1) those cells with greatly contracted chromosomes (considerable despiralization), and (2) those with greatly elongated chromosomes (very little despiralization). Evidently a single bud, or possibly a single anther, contained cells destined to go through division on successive days. Those with the s-chromosomes were to have divided normally on the day that the preparation was made, whereas the cells with the l-chromosomes were to have undergone division the following day; but, owing to a precocious formation of the spindle brought about by the thermal treatment, the chromosomes were advanced a whole day in development, being oriented by the spindle in a partially uncoiled state. It can be stated with some certainty, then, that the effect of the heat in the l-chromosome cells was to accelerate development of the spindle relative to the rate of despiralization, but the exact effect on the s-chromosome cells cannot be so determined. The state of hydration of the chromosomes and spindle probably determines the effect which a high temperature will exert at any particular stage of meiotic development.

Equally striking were the differences in chiasma frequency and distribution between these two cell types (table 2). The l-bivalents probably reveal the approximate position of original chiasma formation, and, as can readily be seen, the chiasmata are largely subterminal. Not infrequently, however, cells are found that show

bivalents with chiasmata formed in the region of the centromere, some of them being extremely close (figs. 3, 13, 14). At normal temperatures never more than two chiasmata per arm have been reported, but these preparations regularly show three in some arms (figs. 3, 4, 13), and rarely four have been found. Seven chiasmata per bivalent has been the highest observed in this material.

Treatment of cells at 40° C. for 24 hours did not markedly increase the total chiasma frequency (table 2), indicating possibly that the l-chromosomes had already undergone chiasma formation at the time of treatment; but the difference in distribution, whether terminal or interstitial, between the s-chromosome and con-

TABLE 2
CHIASMA FREQUENCIES, *T. CANALICULATA*, AT 40° C.

	INTER- STITIAL CHIASMATA	TERMINAL CHIASMATA	UNIVALENTS	TOTAL CHIASMATA
Whole plants grown in greenhouse kept at 22° C. (control).....	4.14	7.37	0.33	11.51
Cut inflorescences kept in oven at 40° C. for 1 day				
Slide no. 1 l-chromosome*.....	8.45	3.21	0.55	11.66
s-chromosome.....	1.96	7.92	1.20	9.88
Slide no. 2 l-chromosome.....	9.70	2.90	0.72	12.60
s-chromosome.....	1.35	8.67	0.80	10.02
Whole plants grown in heat chambers at 40° C. for 2 weeks				
Slide no. 1 l-chromosome.....	9.50	4.20	0	13.70
s-chromosome.....	2.08	6.72	1.28	8.70
Slide no. 2 s-chromosome.....	1.60	7.18	1.33	8.78

* The l- and s-chromosomes are environmental, not genetical, variants and not to be confused with the l-chromosome mutant in *Matthiola* (11).

trol cells and the l-chromosome cells strongly suggests the influence of despiralization on the movement of chiasmata. When plants are grown for 2 weeks at 40° C. (table 2), there is a statistically significant difference in total chiasma frequency between the l- and s-chromosome cells, and—as the increase of univalents would indicate—there seems to have been a loss of chiasmata with contraction. The univalents in these cells probably arise from previously paired chromosomes by a loss of chiasmata. A loss of chiasmata by despiralization is also seen by comparing chiasma frequencies of the l- and s-chromosome cells kept at 40° C. for 24 hours. A comparison of the data on l-chromosomes (40° C. for 2 weeks) with that on l-chromosomes (40° C. for 24 hours) and the controls indicates that chiasma frequency is considerably increased when the entire prophase development takes place at higher temperatures (22, 25).

The l-chromosomes result, as has been stated, from a precocious spindle formation. Under these conditions the appearance of some abnormalities in congression and orientation of the bivalents would be anticipated, since the time allotted for these activities must necessarily be sharply limited. Observations bear this out. In figure 3, one of the rod bivalents has failed to show congression on to the plate, possibly owing to the long and sinuous length of the arms, which would inhibit movement, as well as to a restricted time period, which would necessarily limit the distance through which the chromosomes could move. The arms show no tension, although the bivalent presumably lies within the spindle. At anaphase the arms become extremely long and slender under the tension exerted by the spindle (fig. 4), and the threads appear about to break, as BARBER (1) has shown in heat-treated material of *Fritillaria*. The chiasmata seem unable to terminalize, the strength of union at the point of chromatid exchange being strong enough to resist the separation force of the spindle. The chromosome itself then breaks at the point of greatest stress, this point depending upon its position on the spindle and the position of the most proximal chiasma. BARBER explains that the anaphase tension begins before the chromatid attraction has an opportunity to lapse.

The s-chromosome cells likewise are not without their abnormalities. In some cells the major coils appear unstable, pulling out under the stresses imposed by the spindle, while free arms remain tightly coiled (figs. 2, 5). The chiasmata fail to terminalize, and the threads between the centromere and the first chiasma become unduly stretched, possibly leading to later breakage, although this has not been seen. Figure 5 illustrates the double function of the spindle in effecting chromosome separation. The second bivalent from the left has failed to congress properly on to the plate, with the result that the centromere of the lower homologue occupies a central position on the plate instead of being equidistant from the plate with its homologue. The unoriented homologue behaves as a univalent, independent of its attached partner, and its centromere divides. This behavior is in agreement with SCHRADER'S (19) observations that the centromere is essentially a double structure at metaphase, and its ultimate behavior must therefore depend entirely on its orientation in relation to the plate and not on the singleness or doubleness of its structure. Such behavior was not observed in the case of ring bivalents, for here the bipolarity of the structure as a whole, owing to its two centromeres, more effectively brings about the correct congression and orientation. The pushing action of the upper part of the spindle may be inferred from the appearance of the third bivalent from the right (fig. 5). The chiasma can be seen at the end of the slender stretched arm. No force is being exerted on the homologue, which lies above the chiasma, as evidenced from its unstretched condition. On the other hand, the lower homologue is nearing a point of breakage (arrow) due to the pushing action of the spindle being exerted entirely on a single arm.

It was not possible to follow these two kinds of cells beyond anaphase, so that their second division behavior is unknown. Fragmentation of chromosomes was frequently noticed in those cells containing l-chromosomes. This parallels the observations in *Matthiola* (11), where the *l* mutant, with its long thin chromosomes, showed considerably more fragmentation than did the normal plant. Some underlying mechanism appears to govern chromosome breakage in both instances, reflecting the instability of the l-chromosomes, whether determined genetically (as in *Matthiola*) or environmentally (as in the present instance).

DEVELOPMENT OF MEIOTIC COILS

Little is known of the actual development of meiotic coils from their inception at early stages to their mature proportions at anaphase. The present uncertainty of a universal principle operative in coiling (9, 16) demonstrates the futility of translating unknown prophase stages in *Tradescantia* in terms of known material. However, through the use of the heat treatments described here an experimental tool is available by means of which such difficulties can be overcome, permitting the course of spiralization and despiralization to be traced from incipiency, up to and beyond the normal state of development. The effect of thermal shock was to interrupt the process of meiotic despiralization at various intervals in the course of its development by a precocious or delayed formation of the spindle. This provided a graded series of stages, the integration of which clarifies the prophase coiling cycle. A number of distinct early stages, not at metaphase, add to the observations and facilitate the interpretation.

The coiling cycle during meiotic prophase can be conveniently divided into two stages, the spiralization and the despiralization phases. Theoretically the distinction between the two is obvious, for the first stage is clearly the transformation of an uncoiled structure into one that is regularly coiled, while the second stage is one in which the coils are reduced in number by a process of uncoiling. Actually, however, a sharp delimitation of the two processes is not possible, since—paradoxically—the uncoiling is a continuation of the coiling process, not the reverse of it. That is, if—after a straight thread is thrown into a regular coil—the coiling process continues, not by creating more gyres of the same diameter but by enlarging those already formed, the only means by which this can take place is (1) by elongating the chromonemata, in which case the number of gyres will remain constant; or (2) by allowing the proximal gyres to increase in diameter at the expense of the more distal ones which are progressively uncoiled and lost. The reduction in the number of gyres as prophase progresses indicates that the latter mechanism is operative in *Tradescantia*.

The transformation of the pachytene thread into a coiled structure has been visualized to take the following course. In early diplotene an exceedingly minute coil

makes its appearance. The individual gyres appear as chromomere-like dots on the parallel chromatids (fig. 18), but they can be resolved under the microscope into rather regular coils of equal size and distribution (about $1\ \mu$ or less apart). They are not so closely packed as the later coils but show a relatively greater distance between adjacent gyres. No suggestion of a zigzag or wavy outline prior to this stage was observed in any cells, but from their appearance these minute coils seem to have been "thrown in" on the threads, their initial size being about $0.5\ \mu$ in diameter. Instances were noted where one chromatid showed a change in the direction of the coil not possessed by its sister chromatid, indicating their independence in coiling. The chromatids lie parallel at this early stage (fig. 18), and there is no evidence of chromatid appression (the pressing of the coils of one chromatid into the coils of its sister chromatid so as to give the appearance of a single coiled structure). An attempt was made to determine the number of these small coils at the time of their origin, but the threads could not be followed throughout their entire length.

These minute gyres of early diplotene are destined to become the major coils of metaphase by a process of despiralization. The essential changes taking place during despiralization are three: (1) reduction in chromosome length, (2) reduction in gyre number, and (3) increase in gyre diameter. A fourth change, an alteration in chromonema length (21, 26), may occur, but the *Tradescantia* material was not suited for the detection of such a change if present. The physical basis of despiralization can only be conjectured at this time, although, as has been suggested by numerous cytologists, an explanation might be sought in the behavior of the matrix. In any event, the process of despiralization, with its accompanying changes, is very likely further accompanied by a rotation of the ends of the chromosome arms within the matrix. Where sister chromatids are as intimately coiled as in *Tradescantia*, a rotation of chromosome ends, as the coils decrease in number, is a necessity if entanglement of chromatids is to be avoided, and—as figure 8 shows—little or no entanglement of sister chromatids occurs at anaphase.

The major coils in *Tradescantia* arise as new coils in early diplotene. It seems unlikely, as NEBEL (16) states, that they are homologous with the relic coils of mitosis. In the first place, the pachytene chromosomes appear—in those organisms suitable for favorable study—as completely uncoiled structures, lacking entirely the irregular relic coils associated with somatic prophase. In the second place, *Tradescantia* somatic chromosomes usually have approximately twenty to twenty-five coils. The meiotic coils, if derived from these somatic coils, should be equal in number, but numerous instances of thirty or more coils in the present meiotic material tend to favor a *de novo* origin for the major coils.

By diakinesis the major coils have undergone considerable despiralization, and the chromosome length has been greatly shortened. At this time the minor coil

makes its first visible appearance, becoming increasingly evident as anaphase approaches. The major coils continue despiralization well into anaphase, for anaphase coil counts, taken from the same slides as metaphase counts, are always less in number. There was no evidence of a minor coil at the time of inception of the major coil (fig. 18), and unless it is assumed that the early diplotene threads are submicroscopically coiled, the major coil must precede the minor in chromosomal development. This is contrary to the data of COLEMAN and HILLARY (4), who demonstrate in *Trillium* that the minor coil precedes the major.

The developmental aspects of the minor coil are obscured because of the difficulties of accurate observation, but by a comparison of early and late stages, apparently the same progressive picture obtains as for the major coil; that is, it is one of despiralization following spiralization. Interesting to note, in greatly despiralized chromosomes, is the presence of a subminor coil running perpendicularly to the minor coil. This third minute coil was clearest in those cells in which the major and minor had been relaxed, and although of considerable interest to coiling mechanics, further discussion must await a more detailed study.

The meiotic coiling cycle of *Tradescantia* differs in several ways from the same cycle as seen in *Trillium* (4, 6, 21, 26). HUSKINS and co-workers show that in *Trillium* the number of major coils initially produced is determined by the degree of elongation of the chromonema within a matrix (cf. 4). A comparison of *Trillium* and *Tradescantia* indicates four main coiling differences. The first, relating to the time of development of the major coil as compared with the minor, has already been discussed. The second lies in the great reduction in chromosome length in *Tradescantia* and the lack of chromosome length reduction in *Trillium*, especially during diplotene and diakinesis. The third and fourth differences are concerned with the later development of the major coils. In *Trillium*, once the major coils have been regularly formed, there is no significant change in number of gyres, and there is no significant change in the gyre diameter. The last three differences are interrelated, of course, and if—as has been demonstrated in *Trillium* and assumed for other organisms—the matrix is the morphological unit determining the coiling behavior, it is evident that the matrix in *Trillium* is a more static structure during the time of major coil formation than it is in *Tradescantia*, where the matrix, in order to bound the chromonema, must contract in length and increase in diameter. In *Trillium*, therefore, it appears that the chromonema is the dynamic unit; in *Tradescantia* it is either the matrix, or both matrix and chromonema.

Certain observations point to the type of coiling present in *Tradescantia*, whether of plectonemic or paranemic spirals (21). As already seen, the chromatids coil separately, and the appression of one chromatid coil into that of its sister chromatid occurs as prophase advances. The reticulate appearance of diakinetic chromosomes, as opposed to the single spiraled appearance at metaphase, is evi-

dence of incomplete appression, a phenomenon presumably brought about by progressive dehydration of chromosome and matrix (9). The chromatids can therefore readily separate laterally at anaphase on dissolution of the chromosome matrix, but this reveals nothing of the true nature of the spiral. With relaxation of both major and minor coils (fig. 17), however, it becomes evident that sister half-chromatids can likewise separate laterally, a condition which apparently can result only from the chromonemata being twisted in paranemic spiral; a plectonemic spiral, upon relaxation, would leave the sister half-chromatids relationally coiled about one another. According to SPARROW, HUSKINS, and WILSON (21), a plectonemic spiral of n gyres will give the equivalent of $2n-1$ relational twists if there are no changes in direction. The sister half-chromatids in figure 17 (upper short arrow), however, show only one relational twist, while possessing ten to twelve minor gyres. It has been shown in *Trillium* (21) that the minor coil of the first meiotic division becomes the plectonemic spiral of the microspore division. The difference observed in *Tradescantia* may be real, indicating another point of departure from the *Trillium* type of coiling, or it may be merely apparent, the rotation of the chromosome ends undoing the relational twists and obscuring the true nature of the coils. Further work is needed on this point.

Occasional configurations in heat-treated material are not so readily interpreted in terms of the preceding *Tradescantia*-type coiling cycle (fig. 2). The characteristic small coils are present (fig. 13), but chromosome regions not under anaphase tension seem to be thrown into large, loose, and somewhat unstable coils, as though by adopting these coils the chromosome were being relieved of some internal twist. These coils, in their looseness and irregularity, are unlike the ones in the shortened chromosomes in figures 5, 16, 17, and 19. At this time no explanation can be offered as to their nature or origin, other than that they are heat induced.

A comparison of the development of somatic coils in *Tradescantia* with the meiotic coils reveals certain similarities. KOLLER (8) states that mitotic coiling in the golden hamster proceeds in much the manner described here for the major coils in *Tradescantia*, but NEBEL (16) considers this to be contrary to that found in somatic chromosomes of plants. He states, "The early standard coils appear to be of smaller gyre but not much, if any, more numerous than the final standard gyres." However, KOLLER's observations on animal chromosomes can be readily corroborated in plant chromosomes by a study of the division of the generative nucleus in *Tradescantia* pollen tubes. Here somatic coiling is easily observed, and there is a marked decrease in the number of gyres at metaphase as contrasted with earlier stages (SWANSON, unpublished). The development of somatic coils is similar to that of major coils, and possibly minor coils, in that once the coils are initiated, the remainder of the coiling cycle is occupied by the despiralization process.

Summary

1. The genetical and environmental control of major coiling has been studied in *Tradescantia* from a quantitative point of view. The number of major coils per chromosome was shown to be under complex genetical control.

2. The use of heat treatments revealed that the coiling cycle during meiotic prophase could be broken down into two stages: (1) the initiation of the coils, the spiralization phase; and (2) the loss of coils, the despiralization phase. It was thus possible to show that the major coils of metaphase are derived from numerous coils of small gyre size which are progressively reduced in number by uncoiling at the same time that the gyres are increasing in diameter. Using temperature from 3.5° to 42° C., it was found that at high and low temperatures the despiralization process was accentuated, leading to greater contraction of chromosome length. The greatest number of major coils was found at 27° C. The effect of temperature on the number of major coils was considered both from the standpoint of the despiralization rate and from the time of spindle formation. At 40° C. the coiling was extremely variable, some chromosomes failing to despiralize while others were almost completely uncoiled. Failure of coiling was likewise observed at this temperature.

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LITERATURE CITED

1. BARBER, H. N., The suppression of meiosis and the origin of diplochromosomes. *Proc. Roy. Soc. London B.* 128:170-185. 1940.
2. DARLINGTON, C. D., Recent advances in cytology. 2d ed. Blakiston, Phila. 1937.
3. DARLINGTON, C. D., and LA COUR, L., Nucleic acid starvation of chromosomes in *Trillium*. *Jour. Genet.* 40:185-213. 1940.
4. COLEMAN, L. C., and HILLARY, B. B., The minor coil in meiotic chromosomes and associated phenomena as revealed by the Feulgen technique. *Amer. Jour. Bot.* 28:464-469. 1941.
5. GILES, N., The effect of dehydration on microsporogenesis in *Tradescantia*. *Amer. Jour. Bot.* 26:334-339. 1939.
6. HUSKINS, C. L., and WILSON, G. B., Probable causes of the changes in direction of the major spiral in *Trillium erectum* L. *Ann. Bot. N.S.* 2:281-292. 1938.
7. KAUFMANN, B. P., Chromosome structure in relation to the chromosome cycle. *Bot. Rev.* 2:529-553. 1936.
8. KOLLER, P. C., The genetical and mechanical properties of the sex chromosomes. IV. *Jour. Genet.* 36:177-196. 1938.

9. KUWADA, Y., Chromosome structure. A critical review. *Cytologia* 10:213-256. 1939.
10. LAMM, R., Cytological studies in inbred rye. *Hereditas* 22:217-240. 1936.
11. LESLEY, M. M., and FROST, H. B., Mendelian inheritance of chromosome shape in *Matthiola*. *Genetics* 11:267-279. 1927.
12. MATSUURA, H., Chromosome studies on *Trillium kamschaticum*. I. *Cytologia* 6:270-280. 1935.
13. ———, Chromosome studies on *Trillium kamschaticum*. V. *Cytologia* Fujii Jub. Vol. 20-34. 1937.
14. NAVILLE, A., and DE BEAUMONT, J., Recherches sur les chromosomes des Neuroptères. *Arch. d'Anat. micr.* 29:199-243. 1933.
15. NEBEL, B. R., Chromosome structure in *Tradescantia*. I. *Zeitschr. Zellf.* 16:251-284. 1932.
16. ———, Chromosome structure. *Bot. Rev.* 5:563-626. 1939.
17. SAX, H. J., and SAX, K., Stomata size and distribution in diploid and polyploid plants. *Jour. Arnold Arboretum* 18:164-172. 1937.
18. SAX, K., The effect of variations in temperature on nuclear and cell division in *Tradescantia*. *Amer. Jour. Bot.* 24:218-225. 1937.
19. SCHRADER, F., The structure of the kinetochore at meiosis. *Chromosoma* 1:230-237. 1939.
20. SHIGENAGA, M., An experimental study of the abnormal nuclear and cell divisions in living cells. *Cytologia* Fujii Jub. Vol. 464-478. 1937.
21. SPARROW, A. H., HUSKINS, C. L., and WILSON, G. B., Studies on the chromosome spiralization cycle in *Trillium*. *Canad. Jour. Res.* 19:323-350. 1941.
22. STRAUB, J., Untersuchungen zur Physiologie der Meiosis. VII. *Zeitschr. Bot.* 32:225-268. 1937.
23. SWANSON, C. P., Major coiling and terminalization of chiasmata in *Tradescantia*. *Genetics* 26:172-173. 1941.
24. UPCOTT, M., Timing unbalance at meiosis in the pollen-sterile *Lathyrus odoratus*. *Cytologia* Fujii Jub. Vol. 299-310. 1937.
25. WHITE, M. J. D., The influence of temperature on chiasma frequency. *Jour. Genet.* 29:203-215. 1934.
26. WILSON, G. B., and HUSKINS, C. L., Chromosome and chromonemata length during meiotic coiling in *Trillium erectum* L. *Ann. Bot. N.S.* 3:257-270. 1939.

ANATOMICAL AND PHYSIOLOGICAL RESPONSES OF SQUASH TO VARIOUS LEVELS OF BORON SUPPLY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 536

TAYLOR R. ALEXANDER

(WITH FIVE FIGURES)

Introduction

There are available several summaries of the literature pertaining to boron and plant growth (2, 4, 5, 16, 28). Studies on the internal boron-deficiency symptoms have shown that different species may have certain chemical and anatomical responses in common, but at the same time some variations have been observed. External boron-deficiency symptoms have been found to vary somewhat in time of appearance and in the sequence in which organs are affected—depending upon the species, environment, severity of the deficiency, and age of the plant (3, 7, 16). EATON (6) and others have found that different species grown under identical conditions vary strikingly in the amount of boron absorbed and tolerance to boron concentration, to the extent that plants have been grouped into three categories: sensitive, semi-tolerant, and tolerant. PURVIS and HANNA (19) place squash in the sensitive group and state that in greenhouse soil squash gave a favorable response to borax applications.

In this investigation, squash was selected as the experimental plant in an endeavor to establish its external and anatomical response pattern and to compare it with species previously reported. It was also desired to investigate the degree to which different organs of the plant responded, with particular emphasis on the first visible symptoms. Preliminary experiments, in which squash was grown in boron-deficient solutions, demonstrated the extreme sensitivity of this plant to the absence of boron. Microchemical examination of the tissues indicated a great difference in catalase activity between treated and control plants. No mention of catalase was found in the literature related to boron nutrition, hence catalase determinations were included in the experiments.

Material and methods

The material used in these experiments was the Chicago Warty Hubbard squash, a variety of *Cucurbita maxima* Duchesne. Uniform seeds were selected and planted in fine sand on a greenhouse bench, where the seedlings were grown until they were about 2 inches above the surface of the sand, the cotyledons fully expanded, and the first foliage leaf showing. This stage was reached in about 7

days. At that time the seedlings were carefully removed from the bench by flooding the sand away with a slow stream of water, and they were then washed in several changes of distilled water. Four hundred uniform seedlings were selected and transplanted to $\frac{1}{2}$ -gallon glazed crocks filled with a complete nutrient solution and accommodating five plants per crock. The plants were supported by paraffined sheet-cork in which holes had been punched, the plants being held upright by a light packing of nonabsorbent cotton around the hypocotyls. The crocks were placed on a greenhouse bench and sphagnum moss packed around them. A thin layer of excelsior covering the sphagnum prevented contamination of the nutrient solutions by the sphagnum. This sphagnum-excelsior packing was kept wet during the course of the experiments in an attempt to keep the nutrient solutions as cool as possible. The method reduced the pot temperatures about 10°C . during hot weather.

The solutions contained $\text{Ca}(\text{NO}_3)_2$, KH_2PO_4 , and MgSO_4 at molecular concentrations of 0.006, 0.003, and 0.003, respectively. The minor elements used were manganese as MnCl_2 , zinc as ZnSO_4 , copper as CuSO_4 , and iron as ferric citrate at 0.5, 0.05, 0.02, and 1.0 p.p.m., respectively. Boron was supplied to the control solutions at 0.5 p.p.m. as boric acid. Chemicals were of reagent quality. The pH of the solutions was 4.8–5.0, and the osmotic concentration was approximately 0.7.

All plants were grown on the complete solution (boron included) for 4–5 days, when all solutions were renewed. On renewal, half the plants were changed to solutions without added boron. Roots of the plants placed on boron-deficiency treatment and the crocks containing them had been washed previously with distilled water. The remaining plants continued to receive the complete nutrient solution. The crocks were placed on the bench so that boron-deficient plants alternated with controls in all directions. Iron was added to solutions of both treatments at the rate of 1 p.p.m. every other day, and the nutrient solutions were kept at a given level with daily additions of distilled water. The nutrient solutions were changed again after 5 days.

The experiments were designed to extend 10 days after the boron-deficient series were started, with five harvests, each 2 days apart. Three consecutive experiments, designated I, II, and III, were run during the summer of 1940, and environmental data for each 10-day period were obtained from the U.S. Weather Bureau on the campus. The possible sun hours for the 10-day periods were 146.8, 137.8, and 131.7; actual sun hours were 119.6, 66.0, and 85.8; and the gram calories per square centimeter of horizontal surface were 5392.0, 3648.7, and 3729.4, respectively. Hygrograph records showed the humidity conditions to be similar for all experiments. The floor and walls of the greenhouse were kept wet by frequent sprinkling, and a single thickness of cheese-cloth shade was used when necessary

to prevent wilting. Generally the highest solution temperature recorded each day was around 26° C., but extreme ranges from 23° to 33° C. were noted on the hottest days. Solution temperatures were markedly lower at night.

At harvest, twenty plants were taken at random from each of the two treatments and fresh weights determined. For histological material ten samples were selected from the following plant organs: veins and associated tissue near base of blade, and petioles midway between stem and blade of both the first and second foliage leaves; stem tips; stems just above divergence of first leaf; hypocotyls halfway between the peg and cotyledons; and root tips. Plants in the boron-deficient series ceased appreciable growth after the second leaves had partially developed and the second internode of the stem had elongated. Thus the organs sampled represent the entire treated plant and their control counterparts. Tissues were fixed in Sax's modification of Navashin's solution and stained with a variation of Flemming's triple stain.

Using a modified APPLEMAN (1) apparatus, catalase determinations were made on stem tips including the second leaves, first leaves, hypocotyls, and roots. Duplicate 1-gm. samples, representing twenty plants, were taken from organs which had been cut into small pieces and mixed. Each sample was prepared for analysis by grinding in a mortar for 2 minutes with 1 gm. of CaCO_3 and approximately 1 ml. of water. The ground sample was washed quantitatively into a shaking bottle, a total of 15 ml. of water being used in grinding and transferring. The equivalent of 10 ml. of 3 per cent H_2O_2 (Merck), standardized against KMnO_4 and neutralized to phenolphthalein with $\text{N}/10$ NaOH , was used in each determination. Gas measurements were made immediately after grinding each sample. The shaking bottle was suspended in a waterbath at 25° C., and shaking was started the instant the 10 ml. of H_2O_2 was delivered to the shaking bottle. The time required for the release of either 25 or 50 ml. of gas, depending upon the tissue, was regarded as an index to catalase activity; the time was recorded in seconds from the incidence of shaking.

Results

EXTERNAL SYMPTOMS

In all experiments, boron-deficiency symptoms were identical in aspect and sequence but differed in time of response and extent. Plants in experiment I usually responded 1 day, and in some cases 2 days, earlier than in experiments II and III. Symptoms as they occurred in experiment I are given.

A brittleness in the above-ground portions of the plant, together with a stunted, discolored root system, accentuated in some roots by laterals near the root apex, were symptoms visible on the second day. On the third day general stunting was noticeable, and the second and younger leaves, involving the stem bud, had de-

veloped a yellow spotting and were slightly chlorotic. The second leaves were cupped downward, roughened, and petioles were thickened. Some roots were enlarged near the tips and were of a spongy consistency. By the fourth day spotting

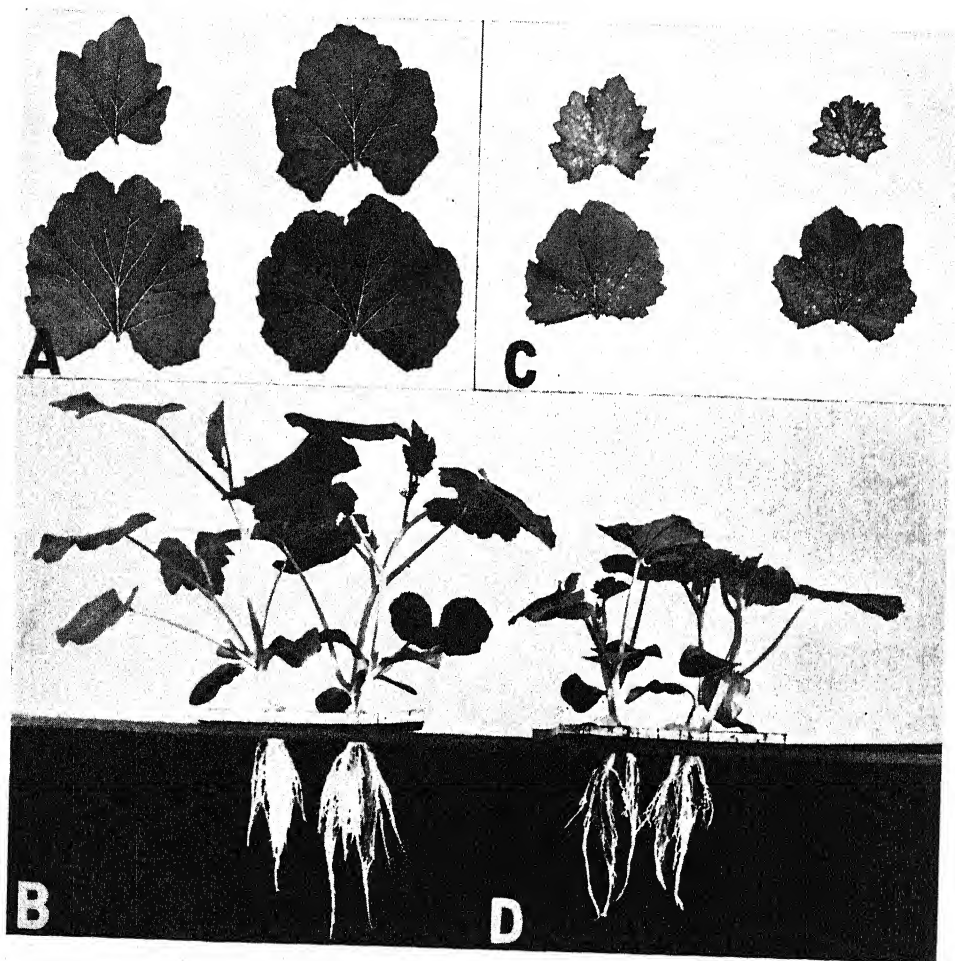


FIG. 1.—External appearance of plants grown 5 days on control and boron-deficient solutions: *A*, second leaves of controls; *B*, control plants; *C*, second leaves of treated plants showing spotting, chlorosis, and stunted size; *D*, boron-deficient plants.

and chlorosis of the young leaves was severe, particularly between the veins toward the base of the blade. Second-leaf petioles and the second internode of the stems were greatly enlarged. A glossy appearance characterized the above-ground portions of the plants (fig. 1). Stem tips had become pale green and soft by the sixth day or were obviously dead.

At the end of the 10-day period all the deficiency symptoms were more pronounced. The control plants had grown until the diameters of their petioles and stems equaled or exceeded those of the boron-deficient plants, which had enlarged rapidly during the first half of the 10-day period. First leaves of the boron-deficient plants remained dark green, stiff, and brittle. The veins of the younger leaves were thickened, contorted, and made prominent by the yellow and chlorotic condition of the vein islets. In all cases, stem tips were dead. Secondary and adventitious roots had succeeded in growing to some extent, but all linear growth had ceased before the experiments terminated. Cotyledons were still green and firm on the boron-deficient plants, whereas they had become wilted and were beginning to fall from the controls.

Plants in the boron-deficient series increased in fresh weight only slightly after the fourth day of treatment, while the controls increased steadily in weight throughout the experiments.

Another difference between the deficient and control plants was their geotropic response. Plants after 2 days in the boron-deficient solutions lagged in their response, while controls responded readily. After 5 days of treatment the boron-deficient plant stems failed to bend, even during a 5-hour exposure to the stimulus.

INTERNAL SYMPTOMS

Internal symptoms described in the following paragraphs were observed in material from experiments I and III, with occasional reference to experiment II. Similar symptoms were found in all experiments, the greatest variation being that plants in experiment I gave evidence of response 1-2 days earlier than plants in experiment II or III. All organs which responded extensively to the deficiency conditions were characterized by progressive thickening of cell walls during the first half of the treatment period, separation of liquefied xylem cells by the enlargement of the xylem parenchyma, and the presence of a red-staining substance of granular appearance in the vessels and tracheids. After the plants had been growing 2 days in the boron-deficient solutions, lignified cell walls of the xylem had a greater tendency to retain violet stain than had comparable tissue of control plants. At a given level in an organ, there were instances in which the same tissue varied in its response from no visible symptoms to extreme symptoms. And in general, boron-deficient tissues tended to mature before similar tissues of control organs.

STEM TIPS.—By the second day, small groups of cells located in the youngest portion of the stem tips were responding in plants of the boron-deficient series, as indicated by their staining qualities; later in the experiment large groups of cells disintegrated in this region. Prior to the fourth-day harvest, unexpanded leaves and leaf primordia had begun to die. In those cases where the primordia

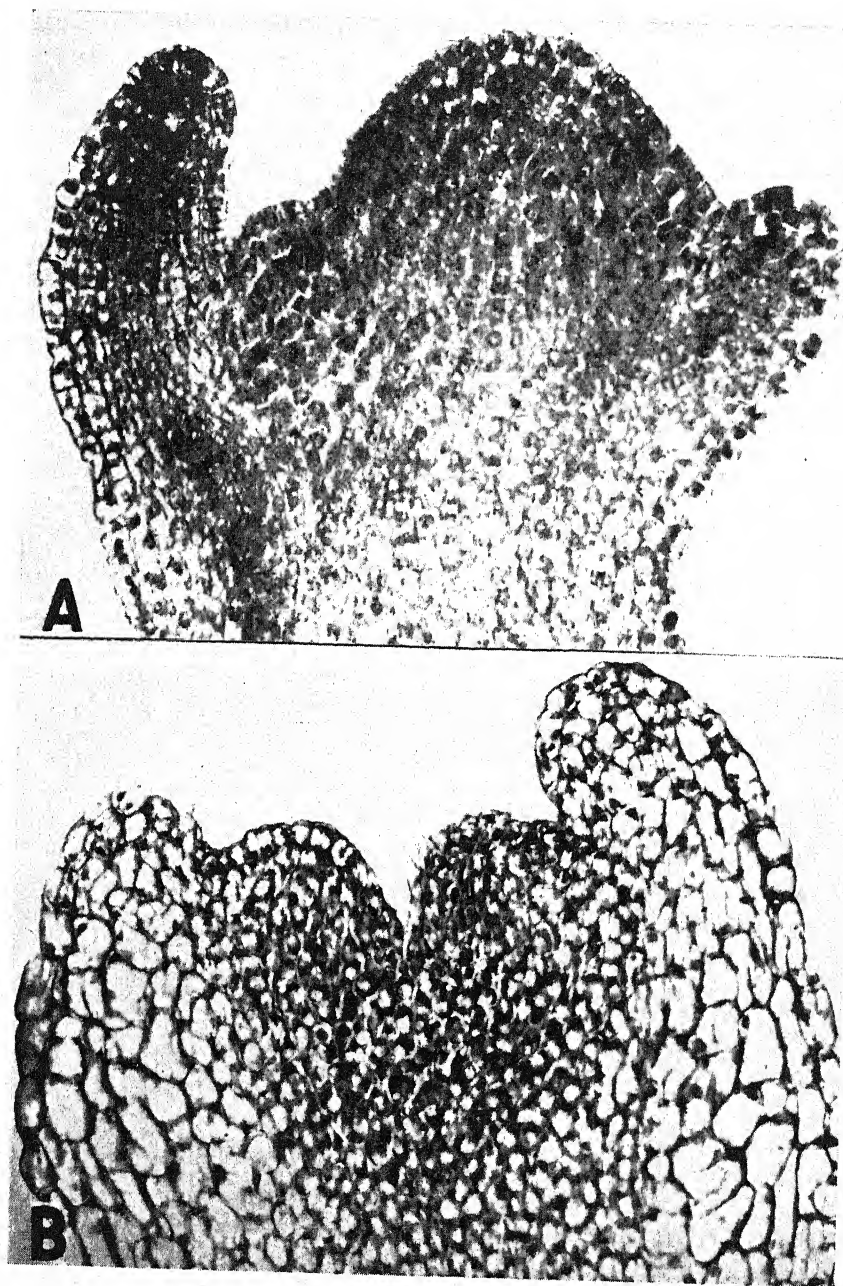


FIG. 2.—Longisections of meristematic regions of stem tip: *A*, control with active embryonic cells; *B*, tip from plant grown 4 days in boron-deficient solutions; cells with disintegrating contents, thickened walls, and frequent cell enlargement; provascular strands not differentiated near apex.

persisted, cells were enlarged, thick walled, and without normal cellular contents (fig. 2). Collapse of the cortical parenchyma, several cells in thickness, had occurred from near the apex to the level of mature protoxylem elements, the dead tissue forming an almost continuous ring just inside the area where a band of collenchyma later differentiates in stems having no boron deficiency. Other cortical

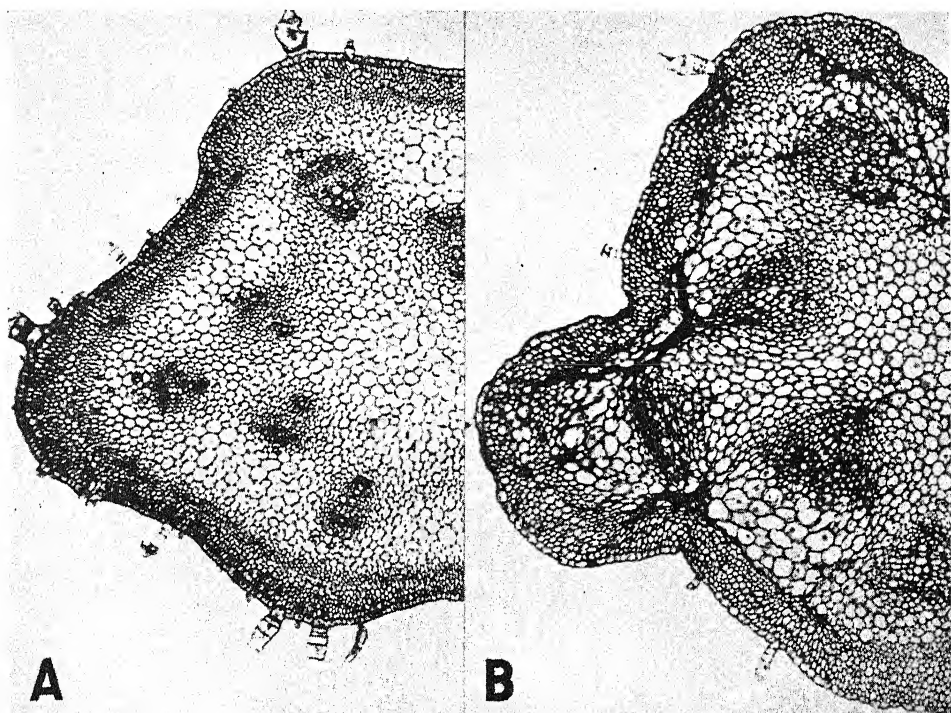


FIG. 3.—Transections from region of maturation in stem tip: *A*, control; *B*, stem from plant grown 4 days in boron-deficient solutions, showing collapse of cells in vascular strands and cortex and enlargement of cortical parenchyma.

cells had thickened walls and were enlarged, whereas cells in the vascular strands and associated parenchyma were dying (fig. 3).

Samples taken on the sixth day showed complete disintegration of large portions of the meristem in the region of greatest cell division. The collapse of the cortical parenchyma, cell enlargement, and disintegration of the embryonic vascular system were more advanced than in former harvests, and in severe cases pith cells were affected. The only additional changes observed on the eighth and tenth days involved death of the tissues at levels progressively farther from the stem apex.

SECOND LEAVES.—Petioles of the boron-deficient plants were larger in diameter than the controls 2 days after treatment was begun. This was attributed mainly to increase in size of fundamental and xylem parenchyma. Collapse of some cortical parenchyma was observed. Plants grown 4 days on boron-deficient solutions possessed petioles which had been further increased in thickness by general hypertrophy of cells of all tissues, especially in the external cambium and the parenchyma between the xylem and the internal phloem. This growth of cells in the vascular bundles had contributed to larger radial and lateral dimensions of the bundles and displacement of the surrounding radially elongated parenchyma, so that in transverse section most bundles were flanked by a group of curved fundamental parenchyma cells. These curved parenchyma cells formed arcs, the centers of which were the vascular bundle (fig. 4). A few tangential divisions had taken place in the lengthened parenchyma cells. Varying amounts of phloem, xylem, and cambial tissue were crushed.

The controls and treated plants had petioles of approximately equal diameter at the end of the experiment. Cell division in the cambiums of the treated plants terminated about the fourth day, whereas the cambiums of the control petioles continued usual development. Increases in size of boron-deficient petioles occurring after the fourth day may be related to the great degree of hypertrophy in the affected tissues.

Marked symptoms had developed in the veins between the second and fourth days. The parenchyma between the adaxial phloem and xylem and the abaxial cambium had elongated in radial dimension, elongation taking place in the order named. This gave rise to a vertically lengthened bundle in which the protoxylem and the most recently formed xylem cells were frequently crushed. Xylem parenchyma cells also became enlarged, as well as the fundamental parenchyma cells adjacent to the bundles. There had been little change in the mesophyll of the boron-deficient leaves since the second harvest. Intercellular spaces were absent, giving the leaf a compact appearance in transverse section. Chloroplasts were small and differed in staining reaction from chloroplasts of control plants.

Progressive changes in the initial symptoms were noted on the sixth, eighth, and tenth days. Greatest increase in cell size had occurred in the abaxial cambium, the cells being radially and laterally enlarged and lacking uniformity of arrangement. The mesophyll cells had grown in size so that they were as large as or larger than the mesophyll cells of control leaves, but there were no intercellular spaces. In contrast, the control leaves were characterized by extensive intercellular spaces.

STEMS.—The stems of the 2-day boron-deficient series were larger in diameter than the control stems and were found to contain enlarged fundamental parenchyma, xylem parenchyma, and parenchyma between the xylem and internal

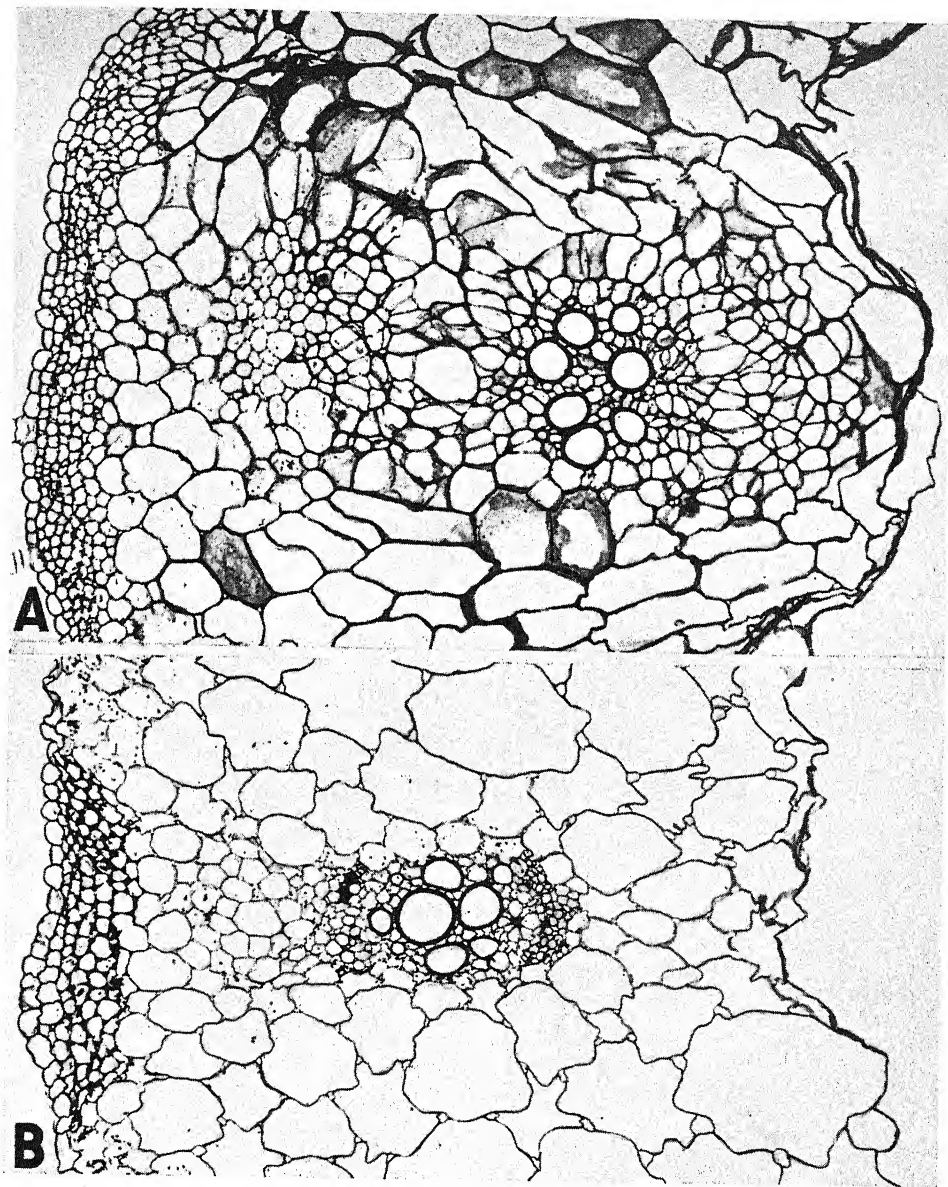


FIG. 4.—Transsection of vascular bundle in second leaf petiole: *A*, bundle from plant after 4 days in boron-deficient solutions; note thickened walls, radially elongated fundamental parenchyma, cambium, and parenchyma cells between xylem and internal phloem, enlarged xylem parenchyma, and crushed cells of cortex; *B*, control.

phloem. Two days later the differences were accentuated by continued cell enlargement of these tissues and the external cambium. External phloem cells and cortical cells immediately outside the bundles in which symptoms were evident were compressed radially. On the tenth day the control stems were of greater diameter than the treated, but on any radius the latter were approximately as

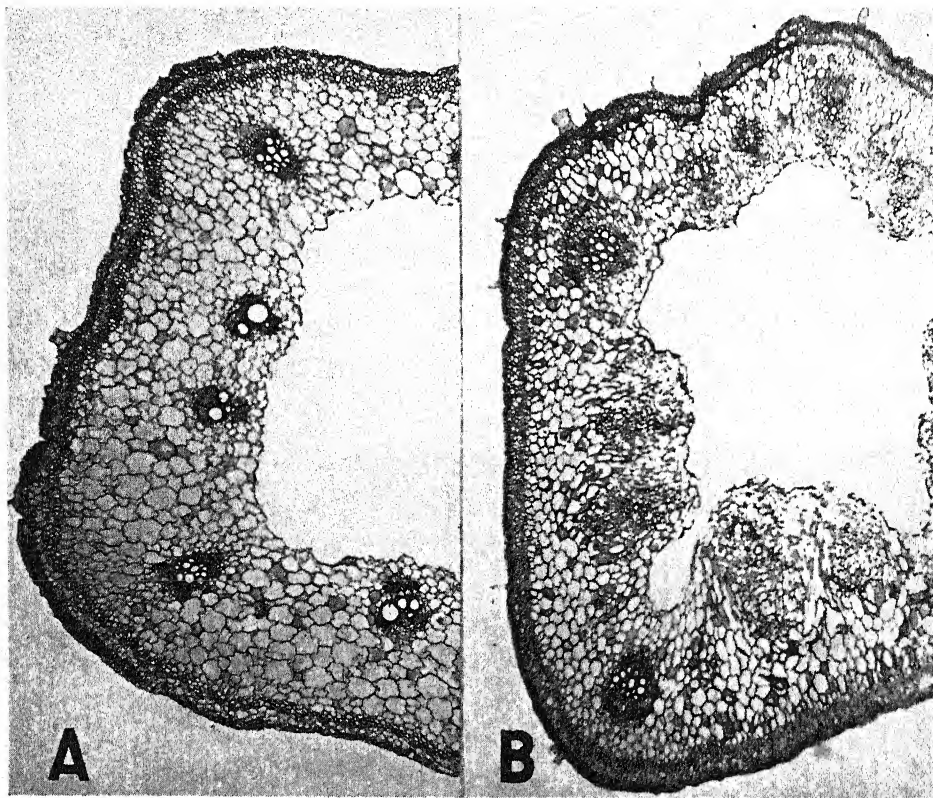


FIG. 5.—Transection of second internode of stem: *A*, control; *B*, stem from plant grown 10 days on boron-deficient solutions; note collapse of cortical parenchyma near upper portion of section and growth of tissue into cavity of stem.

thick from the outer edge of the stem cavity to the outside of the stem. However, unequal centripetal growth of the tissues of treated stems was such that cells in localized areas protruded into the stem cavity, destroying the regularity in thickness observed in the control stems (fig. 5). The bundles which had been largely undifferentiated at the beginning of the treatment consistently had fewer lignified xylem cells than other bundles. These bundles also often contained a group of radially elongated cells located in the region where the external cambium

usually originates. In cortical cells adjacent to or near the ring of sclerenchyma, a collapse of cells which sometimes extended through the collenchyma to the epidermis frequently occurred. These breaks generally described an irregular arc many cells in length (fig. 5).

FIRST LEAVES.—Symptoms of the petioles noted on the last day of harvest paralleled those of the second-leaf petioles. Symptoms made their appearance approximately 2 days later than the latter, however, and were never as great in

TABLE 1
CATALASE ACTIVITY (EXPRESSED IN SECONDS) REQUIRED TO LIBERATE 50 ML. O₂ FOR
STEM TIPS AND FIRST LEAVES AND 25 ML. O₂ FOR HYPOCOTYLS AND ROOTS

ORGAN	SECOND DAY		FOURTH DAY		SIXTH DAY		EIGHTH DAY		TENTH DAY		SIGNIFICANT DIFFERENCES	
	+B	-B	+B	-B	+B	-B	+B	-B	+B	-B	1%	5%
EXPERIMENT II												
Stem tips....	10.3	8.5	15.6	4.8	20.1	6.7	15.1	6.9	1.9	1.3
First leaf....	26.6	18.5	30.3	26.0	51.0	27.6	45.3	28.5	3.2	2.2
Hypocotyl....	94.0	136.0	66.0	50.5	102.1	81.8	139.0	39.7	32.3	22.2
Roots.....	33.3	58.4	25.0	33.0	37.0	40.9	47.6	41.5	14.9	10.3
EXPERIMENT III												
Stem tips....	10.3	6.3	20.5	8.9	23.9	6.1	13.6	4.5	11.1	8.7	7.0	4.9
First leaf....	7.5	6.5	15.3	8.7	15.5	6.5	9.4	5.7	11.7	3.9	1.8	1.3
Hypocotyl....	64.0	56.7	91.5	73.0	132.0	48.0	75.0	24.0	54.0	10.0	25.7	18.1
Roots.....	92.0	81.3	101.0	85.0	88.0	43.0	91.0	56.5	81.0	44.0	10.5	7.4

extent. The mesophyll of leaves from the two treatments was similar in all visible aspects.

HYPOCOTYLS.—As a rule, material from all three experiments failed to show significant differences between the treated and control plants. But an occasional bundle was observed in which there was a suggestion of initial boron deficiency, as had occurred earlier in stem bundles.

ROOT TIPS.—Description of boron-deficiency symptoms in roots is difficult since varying degrees of response could be observed in material of a given harvest. Incipient and advanced symptoms were evident on the second day of treatment. Contrasted with control roots, the histogen region of boron-deficient roots had decreased in extent, and maturation processes were active nearer the apex. At levels where there had been no differentiation of stelar tissues, collapsed, disinte-

grated, and enlarged cells or groups of cells were observed in the peripheral portions of the stele. Hypertrophy of the embryonic stelar tissue and stretching of the slightly enlarged cortical cells by growth of stimulated lateral root primordia which were prominent almost to the tip of some roots, were factors in the increase of root diameter.

Roots examined after 4 days in the boron-deficient solutions were affected severely. Death and disintegration of the extremities of the tip were not uncommon. In the region of elongation and maturation, individual cells were enlarged, others were crushed, and in some roots the central portion of the stele was so completely disintegrated that cells could no longer be recognized. Farther from the apex there was crushing and collapse of cells associated with growth of laterals, and frequently the lateral roots developed deficiency symptoms before emerging from the cortex. These symptoms were characteristic of those found on the remainder of the harvest days, except that deterioration became more extensive.

CHEMICAL MEASUREMENTS

The data on catalase determinations may be found in table 1, where the figures for catalase activity are averages of duplicate samples. The "significant differences" included in the table refer to differences necessary for significance between the control organs and the treated ones on any harvest date. In the cases of stem tips and first leaves, the evolution of oxygen from preparations of boron-deficient tissue was so rapid that it was found necessary to use 50-ml. volumes. Hypocotyl and root tissues were relatively inactive; therefore the volume was reduced to 25 ml. when these tissues were analyzed.

Discussion

Differences in time required for the appearance of both external and internal symptoms of deficiency in experiment I as contrasted with experiments II and III have been mentioned. WARINGTON (26) reported that the delay in appearance of symptoms in other seasons compared with summer is correlated with decreased day length. EATON (7) also mentions variations in response between seasons and in growth periods within seasons. Experiment I was conducted under comparatively high light and temperature conditions, and this probably accounts for the discrepancies noted between experiments. The effect of external conditions on catalase activity was not ascertained, since such activity was determined only for experiments II and III, which were conducted during periods of comparable conditions.

In squash the response to boron-deficient nutrition was found to be general throughout most of the plant. All organs responding extensively by the second day had developed entirely or in part after the treatment was started, and the relative maturity of the hypocotyls and first leaves at the beginning of the treat-

ment might have been a factor in limiting their response. Stem and root tips, second leaves, and stems gave anatomical evidence of response within 2 days. Of these, the apical meristems were the most reactive. Disintegration and collapse of parenchyma cells in the apex of the meristem and cortex of the older portions were the first responses in the stem tips; while hypertrophy of xylem parenchyma, fundamental parenchyma, and cambium characterized the earliest symptoms in leaves and stems. The downward cupping of the second leaves after the plants had been growing in boron-deficient solutions 3 days can be correlated with the first visible internal symptom; namely, the increased size of parenchyma cells between the xylem and the internal phloem. In petioles and stems, likewise, comparable tissue tended to be the first to increase in size. The paucity of intercellular spaces in the mesophyll of second leaves under the treatment was probably associated with the failure of these leaves to expand fully.

Hypertrophy and disintegration of the cambium followed by disintegration of the phloem has been reported as an early symptom in stems of *Vicia faba* (25), citrus (9), and sugar beet (21). The short duration of the experiments in this investigation might account for the relatively limited response of the phloem. Ground parenchyma is also frequently affected, as shown by sugar beet (2, 21) and *V. faba* (25). DENNIS (4) quotes work of JAMALAINEN, in which the first symptom of deficiency in Swedish turnip was enlargement of the xylem parenchyma. The collapse of cortical parenchyma in the cortex of the stem and petiole of squash may be similar to the conditions preceding "cracked stem" of celery caused by boron deficiency (20).

In the root tip the stele was the region which responded most to the deficiency. Hypertrophy of the cells and stimulated lateral root primordia were the main symptoms. These results are in general agreement with WARINGTON's report on *V. faba* (25) but differ in some aspects from work reported by SOMMER and SOROKIN (23). The latter, working with *Pisum sativum*, found the outstanding symptoms to be hyperplasia of the plerome, hypertrophy of the periblem, and abundant primordia of secondary roots near the apex. ROWE (21) stated that sugar-beet roots merely failed to elongate in boron-deficient solutions. The fact that varying degrees of response in root tips were observed during the early phases of the present experiments has been mentioned previously in the account of internal symptoms. Some of these differences may be related to variation in response between individual plants. It was noticed after treatments were started that the boron-deficient plants frequently developed adventitious roots from the hypocotyl. These roots grew rapidly for a short time, while other roots had ceased terminal growth. The adventitious roots were occasionally sampled with the other roots, and this might also have been partially responsible for some variations seen in anatomical symptoms between and within harvests.

With the exception of the first harvest date, significant differences in catalase activity of the stem tips and first leaves were obtained between the treated and control plants. The data (table 1), however, show that on the first harvest, differences were either significant or approaching significance at 5 per cent. Samples prepared from the treated tissues were always more active than those from control tissue in the liberation of gas, the greatest difference occurring about the sixth day. At this time the treated tissues required only about one-third the time that control tissue did to liberate equal quantities of oxygen. Tissues containing the greatest number of chloroplasts were most active. Besides liberating gas slowly, hypocotyls and roots usually gave less consistent results between experiments and harvest dates within experiments. On the other hand, the activity of these boron-deficient organs consistently exceeded the controls on each harvest day of experiment III. The use of entire roots—rather than the root tips—might account in part for their comparative lack of activity. With the exception of root tips, there was a positive correlation between the organs with the first and most extensive anatomical response and those with the highest rate of catalase activity.

The function of catalase in the plant cell has not been demonstrated, although there are numerous reports on the relation of catalase activity to respiration and general metabolism. HEINICKE (14) contended that catalase activity is a measure of the metabolic status of tissues and that it might serve as an indicator of the physiological responses of plants to their environment. Considering the occurrence of abnormal growth and disintegration of cells in tissues studied, the following reports are more specific and possibly of correlative nature. HARVEY (13) found that "overgrowths" produced by freezing or by *Bacillus tumefaciens* on *Ricinus*, beet, and *Bryophyllum* brought about a lower pH of tissue and increased the activity of catalase over normal tissue. Later it was shown that potato tissue affected with the potato wart disease, which also caused "overgrowths," had twice the catalase activity of normal plant tissue (27). A physiological disorder of apples in storage known as "soggy breakdown" is preceded by and associated with high catalase activity, as contrasted with normal apples (11, 18). With reference to enzymatic activity and boron nutrition, only one report was found. HAAS and KLOTZ (10) reported that citrus leaves from boron-deficient plants exhibited a slightly greater diastatic activity than normal leaves.

The catalase molecule has received considerable attention in recent years. Catalase is now thought to be a chromoprotein composed of a colored prosthetic group, apparently a hematin compound, with a protein (8). Hematin is an iron-porphyrin compound (12), and the family of iron-porphyrin proteins includes others of the most active catalysts known, for example, peroxidase and cytochrome. There is general agreement that the iron present in catalase is in the ferric state, and the number of iron atoms per molecule is considered to be four

(8). ZEILE and HELLSTRÖM (30) demonstrated positive correlation between enzymatic activity and content of porphyrin-bound iron in catalase from horse liver. Other workers (24) have found that those preparations of horse and beef liver catalase which are the most active produce the greatest quantity of hemin iron.

ZEILE (29) investigated catalase prepared from cucurbit seedlings (Samen der Kurbissorte "Lang gul Märg") and found that it gave absorption bands in common with catalase from horse liver; he concluded that plant and animal catalase are similar. This work was confirmed by KEILIN and HARTREE (15), who prepared their catalase from germinating cucumber seeds.

Examination of the symptoms preceding and coexistent with the increased catalase activity in the boron-deficient plants and the chemical structure of catalase suggested some possible correlations between the extremely high catalase activity and the chlorotic condition of the most active tissues. Chlorophyll has a chemical structure related to the porphyrins, and the present concept of the chlorophyll molecule has such a nucleus (12). Hence, the destruction of chlorophyll or interference with its normal synthesis could lead to the accumulation of compounds related to the prosthetic group of catalase. NEISH (17), reporting on three species of plants, stated that most of the catalase in leaf cells is found in chloroplasts together with a concentration of iron and copper—all of which are oxidative catalysts. The leaves which developed under the boron-deficient treatment failed to develop normal chloroplasts. Further, the necessity of iron for the formation and maintenance of chlorophyll has been established (16), and SHIVE (22) states that functional iron is in the reduced state. If it is assumed that the increased catalase activity represents a larger percentage of total iron in the ferric state than in less active tissues, the inactive condition of the ferric iron might account for some of the chlorosis observed in the boron-deficient squash.

The brittleness of the boron-deficient tissue suggested another possible explanation for the increased catalase activity. It was thought that the brittle tissue might grind more readily and bring about a greater extraction of the enzyme. However, variation in the time of grinding brought about no significant differences in results. The influence of the affected chloroplasts as a factor was not investigated.

Summary

1. Plants were grown 10 days on control and boron-deficient solutions. Samples representing the entire plant were taken every second day for anatomical examination and catalase determinations.
2. External symptoms of boron deficiency were described for the entire plant and were correlated where possible with internal symptoms.
3. Anatomical responses were evident in stem and root tips, second leaves,

and stems 2 days after the boron-deficient treatment was started. In the stem tips, cell enlargement and collapse occurred in the region of cell division and in the cortex of more mature portions. The stelar cells of the root tip were similarly affected. Tissues which were actively growing at the beginning of the treatment were the most responsive. Hypocotyls were very inactive.

4. Hypertrophy of the xylem parenchyma and the parenchyma in the regions where the internal and external cambiums usually originate was the first visible response in organs with differentiated vascular bundles.

5. Boron-deficient organs showed more catalase activity than control organs, the differences being obvious even on the second day of treatment. With the exception of root tips, the greatest catalase activity was measured in the organs with the earliest and most extensive histological responses. These organs were also the ones which became chlorotic, and a possible correlation between this condition and the extreme catalase activity was suggested.

6. Plants of the boron-deficient series soon lost their capacity for geotropic response. The first evidence of this was noted on the second day of treatment.

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LITERATURE CITED

1. APPLEMAN, C. O., Some observations on catalase. *BOT. GAZ.* 50:182-192. 1910.
2. BRANDENBURG, E., Über die Grundlagen der Boranwendung in der Landwirtschaft. *Phytopath. Zeitschr.* 12:1-112. 1939.
3. CHANDLER, F. B., Boron deficiency symptoms in some plants of the cabbage family. *Maine Agr. Sta. Bull.* 402. 1940.
4. DENNIS, R. W. G., The relation of boron to plant growth. *Science Progress* 32:58-69. 1937.
5. DENNIS, R. W. G., and O'BRIEN, D. G., Boron in agriculture. *West Scot. Agr. Coll. Res. Bull.* 5. 1937.
6. EATON, F. M., Boron in soils and irrigation waters and its effect on plants. *U.S. Dept. Agr. Tech. Bull.* 448. 1935.
7. ———, Interrelations in the effects of boron and indoleacetic acid on plant growth. *BOT. GAZ.* 101:700-705. 1940.
8. ELVEHJEM, C. A., and WILSON, P. W., *Respiratory enzymes*. Burgess Publishing Co., Minneapolis. 1939.
9. HAAS, A. R. C., and KLOTZ, L. J., Some anatomical and physiological changes in citrus produced by boron deficiency. *Hilgardia* 5:175-197. 1931.
10. ———, Further evidence on the necessity of boron for health in citrus. *BOT. GAZ.* 92:94-100. 1933.
11. HARDING, P. L., Relation of catalase activity to temperature, respiration, and nitrogen fertilization of Grimes Golden apples. *A.S.H.S.* 27:37-42. 1930.
12. HARROW, B., *Textbook of biochemistry*. Philadelphia. 1940.
13. HARVEY, R. B., Relation of catalase, oxidase, and H-ion concentration to the formation of overgrowths. *Amer. Jour. Bot.* 7:211-221. 1920.

14. HEINICKE, A. J., Factors influencing catalase activity in apple leaf tissue. New York Agr. Sta. (Cornell) Mem. 62. 1923.
15. KEILIN, D., and HARTREE, E. F., On some properties of catalase haematin. Proc. Roy. Soc. London B. 121:173-191. 1936.
16. McMURTREY, J. E., JR., Distinctive plant symptoms caused by deficiency of any one of the chemical elements essential for normal development. Bot. Rev. 4:183-203. 1938.
17. NEISH, A. C., Studies on chloroplasts. II. Their chemical composition and the distribution of certain metabolites between the chloroplasts and the remainder of the leaf. Biochem. Jour. 33:300-307. 1939.
18. NELLER, J. R., Relation of catalase activity to physiological breakdown in Jonathan apples. Plant Physiol. 6:347-354. 1931.
19. PURVIS, E. R., and HANNA, W. J., Vegetable crops affected by boron deficiency in eastern Virginia. Virginia Sta. Bull. 105. 1940.
20. PURVIS, E. R., and RUPRECHT, R. W., Cracked stem of celery. Florida Agr. Sta. Bull. 307. 1937.
21. ROWE, E. A., A study of heart-rot of young sugar-beet plants grown in culture solutions. Ann. Bot. 50:735-746. 1936.
22. SHIVE, J. W., Significant roles of trace elements in the nutrition of plants. Plant Physiol. 16:435-445. 1941.
23. SOMMER, A. L., and SOROKIN, H., EFFECTS of absence of boron and of some other essential elements on the cell and tissue structure of root tips of *Pisum sativum*. Plant Physiol. 3:237-260. 1928.
24. SUMNER, J. B., DOUNCE, A. L., and FRAMPTON, V. L., Catalase. III. Jour. Biol. Chem. 136:343-356. 1940.
25. WARINGTON, K., The changes induced in the anatomical structure of *Vicia faba* by the absence of boron from the nutrient solution. Ann. Bot. 40:27-42. 1926.
26. ———, The influence of length of day on the response of plants to boron. Ann. Bot. 47:429-457. 1933.
27. WEISS, F., and HARVEY, R. B., Catalase, hydrogen-ion concentration, and growth in the potato wart disease. Jour. Agr. Res. 21:589-592. 1921.
28. WILLIS, L. G., Bibliography of references to the literature on the minor elements and their relation to plant and animal nutrition. 3d ed. Chilean Nitrate Educational Bureau, New York. 1939.
29. ZEILE, K., Über die aktive Gruppe der Katalase. II. Zeitschr. Physiol. Chem. 195:39-48. 1931.
30. ZEILE, K., and HELLSTRÖM, H., Über die aktive Gruppe der Leberkatalase. Zeitschr. Physiol. Chem. 192:171-192. 1930.

ROOT DISTRIBUTION AND ENVIRONMENT IN A MAPLE-OAK FOREST

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 537

NORBERT J. SCULLY

(WITH TWENTY-ONE FIGURES)

Introduction

Various studies have emphasized the importance of investigating root distribution (*a*) as a phase of the structure of communities; (*b*) in correlation with horizontal characteristics of soil profiles which might directly or indirectly affect root growth; (*c*) as a background for the logical sampling of soil factors; (*d*) in interpreting plant competition studies and individual plant responses. All these outlooks are necessary in a complete analysis of environment and community relationships. The present study was approached primarily from the first two points of view but also furnishes a background for further work along other lines.

The trench method of investigating roots (18) offers no difficulty when most roots present belong to one or a few dominant species. In deciduous forests with luxuriant ground cover, however, difficulties in root identification tend to limit the value of such study to simple correlation based only on the total root distribution, although many of these difficulties can be overcome by certain modifications and additions to the method.

Investigation of root distribution by the trench method is essentially statistical. Any method employed should measure accurately the variability in the population to be sampled. A refinement of methods to cope with the degree of heterogeneity is desirable. The present investigation offers a partial solution to the problem in deciduous forests, where roots are numerous. Further, it seems desirable that any study of relationship between root distribution and environment should consider the whole biology of the soil profile. Such study demands analysis of the soil proper; of inherent growth tendencies of the root systems of given dominant species as well as their responses to specific soil characters; and—of equal importance in most habitats—the role played by soil invertebrates and burrowing animals. Animal activities are in need of much more study, particularly in regions exhibiting the mull type of forest soil profile.

The present study consists primarily of a root distribution and environment investigation in a 65-acre tract of sugar maple-red oak-white oak forest. Counts were made of all roots occurring in profiles of sixteen selected stations, employing an old and a new method of trench sampling; and an attempt was made to cor-

relate their occurrence in number, size, and area to the respective horizontal features. A number of physical soil factors and the occurrence and role of earthworms and cicada nymphs in the profiles were observed.

The investigation was conducted during the summers of 1938, 1939, and 1940 at Wychwood, Lake Geneva, Walworth County, Wisconsin.

Regional habitat conditions

CLIMATE

Nearly all of Walworth County is included within the Rock River basin. This climatic province has the longest growing season of any in Wisconsin, averaging approximately 170 days (20). The mean annual temperature at the Weather Bureau Station at Delevan, a few miles from Wychwood, is 46.0° F. The average annual precipitation of 31.4 inches is distributed rather uniformly throughout the growing season, with an average rainfall of more than 2.6 inches per month from April to September, inclusive. The data from the Delevan station indicate comparatively slight climatic fluctuation.

PHYSIOGRAPHY

Wychwood lies on the Darien moraine of the "kettle-range," one of the most striking topographic features of a large portion of eastern Wisconsin (17). About Lake Geneva, on the north shore of which Wychwood is located, the topography is typically irregular. Within the 65 acres of forest a definite knob-and-kettle topography is found, although there are only a few true kettles. Slopes of 15°–20°, occasionally extending 100 feet or more, are comparatively common. The land rises to the north in the $\frac{2}{3}$ -mile length to a maximum of 1029 feet above sea level, or 168 feet above the lake, affording good conditions for drainage. A few depressions without surface outlets occur, but they normally possess comparatively good internal drainage.

SOIL

All profiles examined at Wychwood correspond to the Bellefontaine silt loam type (8, 11, 17), a characteristic type on the more rolling till deposits in eastern Wisconsin, belonging to the Gray-Brown Podsollic Group. The underlying rock formation, from which the glacial deposits at Wychwood are derived in large part, is Niagara limestone. Owing to the calcareous nature of the parent soil material and the annual return of large supplies of bases to the surface through the decomposition of fallen leaves, a "coarse mull" type (7) of soil has developed in which the organic matter is intimately mixed with the upper few inches of the mineral soil.

The A₀ horizon is variable in thickness because of the irregular local topography

and because the annual leaf fall completely disappears, except for occasional petioles, before the next year's foliage is shed. The rapidity with which leaves decompose in this forest during the summer months is unusual. The possible role of earthworms (*Lumbricus* spp.) in this regard is treated later.

The profiles examined typically had a dark gray, silt loam A₁ horizon with an average depth of 4 inches. With few exceptions, the horizon is relatively high in organic matter in the specific stations and in other sampled areas. Numerous small but visible pores were evident, and the soil was distinctly crumbly and friable.

The A₂ horizon, occasionally absent, was a grayish yellow silt loam, indication that leaching had not been complete. It was somewhat lighter in texture than the A₁. Encountered at an average depth of 4 inches, it had an average thickness of 5 inches. Typically this horizon was the lightest colored in the profile and showed a slight blocky structure at its lower levels.

The B₁ horizon, a heavy silt loam, occasionally with varying amounts of sand, varied in color from dull brown to yellowish brown. Encountered at 9 inches, it was 4 inches thick. The soil had a well-marked, small blocky structure which was more evident than in the A₂.

The B₂ horizon, encountered at 13 inches, was 19 inches thick and consisted of a heavy, reddish brown sandy clay. It had a characteristic angular nut structure which became more massive as the C horizon was approached. At the upper limits of the C₁, the nut structure disappeared. Upon wetting, the B₂ horizon becomes very sticky and plastic. Upon drying, the individual blocks shrink and the sutures give rise to marked hair-line crevices. Isolated white to light yellow particles of cherty material of variable size characterized the horizon, particularly in the lower portions.

Appearing at a depth of 32 inches, the C horizon consisted of a gravelly sandy clay or a gravelly sandy loam. Limestone rocks were found in variable numbers and sizes in the upper 6-inch level of the horizon.

This soil type is subject to erosion, evidenced by exposure of the A₂ and, occasionally, B₁ horizons in scattered areas. Erosion is not confined to areas with marked slopes but also occurs on comparatively level surfaces.

Vegetation

Deciduous forest is the climatic climax in the region. In the southeastern portion of Walworth County it was originally represented by a mixture of several species of oak, maple, hickory, etc. (17). At present the forest at Wychwood, one of the few relatively undisturbed stands in the region, is primarily sugar maple-red oak-white oak. The older oaks are more than 200 years old, while many maples are 100 years or more.

TABLE 1

RELATIVE ABUNDANCE* AND FREQUENCY INDEX OF STATION SPECIES

SPECIES	STATION†																FRE- QUENCY INDEX (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
A. Herbaceous:																	
<i>Actea alba</i>						R							R			R	19
<i>Adiantum pedatum</i>			R														6
<i>Amphicarpa bracteata</i> (p)†.....						R		C	C		R	A		R			38
<i>Aralia nudicaulis</i> (p).....				C	R			R	C		C	C	R	C	C	R	56
<i>Arisaema triphyllum</i> (p).....	R	C	C			C	R	C	C	C		C	C	R	C		69
<i>Aster</i> sp.....						C		R				R	C	R	C	C	50
<i>Carex pennsylvanica</i> (p).....				A	A	R						A	C	R	R	C	50
<i>Caulophyllum thalictroides</i> (p).....	R	R		R			R	C	C	R						C	50
<i>Circaea latifolia</i>						C										C	13
<i>Cryptotaenia canadensis</i>						C											6
<i>Cypripedium parviflorum</i>												R					6
<i>Desmodium strictum</i> (p).....				R							R	R	R		C	R	38
<i>Dioscorea villosa</i>	R							C	R		R	C	R				38
<i>Eupatorium purpureum</i> (p).....						C			R	R					R	R	31
<i>Eupatorium rugosum</i> (p).....				R	R			C	R						R	C	38
<i>Galium</i> sp.....													R	R	R	R	25
<i>Geranium maculatum</i> (p).....			R	R	R	C	R	C	R		R	C			C	C	69
<i>Geum</i> sp.....							R										6
<i>Hepatica acutiloba</i> (p).....						C		R	C		R	R	R	R			44
<i>Helianthus</i> sp.....					R						R						13
<i>Laportea canadensis</i> (p).....						R											6
<i>Menispermum canadense</i> (p).....						C											6
<i>Osmorhiza claytoni</i>				R													6
<i>Phryma leptostachya</i> (p).....						C											6
<i>Podophyllum peltatum</i> (p).....	R	R	R			C	R	R	R	R	C	C	R			C	75
<i>Polygonatum pubescens</i> (p).....			R	R	R			C	C					R	C		44
<i>Prenanthes</i> sp.....	R			R									R				19
<i>Ranunculus septentrionalis</i> (p).....									R		R			R			19
<i>Sanguinaria canadensis</i>			R					C						R			19
<i>Sanicula</i> sp.....						R											6
<i>Smilacina racemosa</i> (p).....		C	C		C		R	C	C	C	C	R	R	R	C	C	81
<i>Smilax ecirrhata</i> (p).....	R	R	R					R	R		R				C		44
<i>Solanum dulcamara</i> (p).....						R											6
<i>Solidago latifolia</i> (p).....	R	R	C	C	R		C	C	C	C	C	C	C	R	C	C	94
<i>Solidago</i> sp.....				R	R					R	R	C	R	R	C		50
<i>Thalictrum dioicum</i> (p).....									R	R					C		19
<i>Trillium grandiflorum</i>						R		R					R				19
<i>Trillium recurvatum</i>								R									6
<i>Uvularia grandiflora</i> (p).....						C	R	C	C	C	R			R	C		50
<i>Viola</i> sp.....																C	6
No. of species per station.	7	6	10	11	9	17	8	17	17	9	18	14	13	13	18	12

* Relative abundance: A, abundant; C, common; R, rare.

† Stations 1-10 sampled in 1938; stations 11-16 sampled in 1939.

‡ (p), one or several plants or their root systems photographed in 1938-1940.

TABLE 1—*Continued*

SPECIES	STATION†																FRE- QUENCY INDEX (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
B. Woody:																	
Acer saccharum (p).....	A	A	A	C	C	C	A	C	A	A	A	A	A	A	A	A	100
Amelanchier canadensis.....								C			R						25
Carya cordiformis.....			R		C	C	C		C				R	R	C	R	56
Cornus alternifolia.....			C									R			R		19
Fraxinus americana (p).....	C		C	C	C	C		R	C	C	C	C	C	A	A	C	88
Fraxinus nigra.....								R				R					13
Hamamelis virginiana.....			R									C					13
Lonicera dioica (p).....	C			C							C	C	A	R	R		44
Ostrya virginiana.....		C	R	C	C			C	C	C	C	C	C		C	C	75
Parthenocissus quinquefolia (p).....			C	R	R	C	A	C	C	R	C	R	R		A		75
Prunus serotina.....	R		R	R							R			C			31
Prunus virginiana (p).....	C	C	C	C			C	C	C	C		R		C	R	C	75
Quercus alga (p).....		C	R	C	C	C	C	C	R		A	A	C	C	C	C	88
Quercus borealis maxima (p)	C	A	C	A	A	C	C	A	C	R	C	C	A	A	A	C	100
Rhus radicans.....																C	6
Ribes cynosbati.....													C	R			13
Tilia americana.....	C	C					C		C	C	C		A	C	C	C	63
Ulmus americana (p).....		R							R			C			R		25
Ulmus fulva.....	C		R									C	C	C	C	C	44
Viburnum acerifolium.....				C	A					C	C	C	C		R	R	50
Vitis sp.....											R					C	13
Zanthoxylum americanum.....	C	R					C				R		R				31
No. of species per station.	9	8	12	10	8	6	8	8	10	8	14	13	15	12	14	12

A detailed cover or frequency analysis of the vegetation was not made.¹ Table 1 shows the presence, relative abundance, and frequency index of species found, based on circular areas 25 yards in diameter around each trench.

The stations were designated numerically, since they could not be differentiated individually by any one characteristic. While it would be desirable to classify the sixteen stations on the basis of vegetation, it was impractical and confusing since their floristic composition was not sufficiently variable and other factors in the selection of a station were slighted unwarrantedly.

Investigation

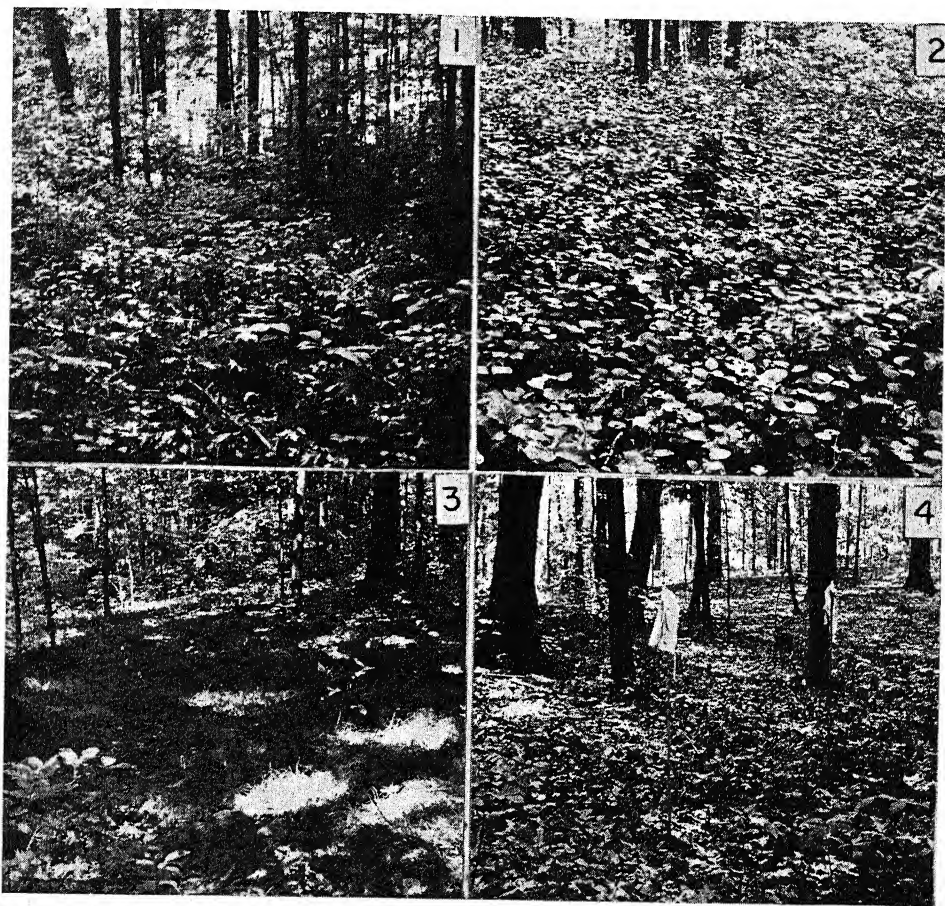
GENERAL METHODS

The sixteen stations were selected on the basis of relative abundance and type of herbaceous and woody ground cover, nature of soil, and topographical situation. A cross-sectional sample of conditions within the forest was thus obtained.

¹ DR. CHARLES E. OLMSTED, under whom this study was completed, has been conducting an investigation of the environment of this forest for a period of 6 years. The data, when published, will provide a treatment of the floristic composition.

Those station types which occupied the larger areas were sampled several times to assure reasonably accurate measurement of their root distribution and development (figs. 1-4).

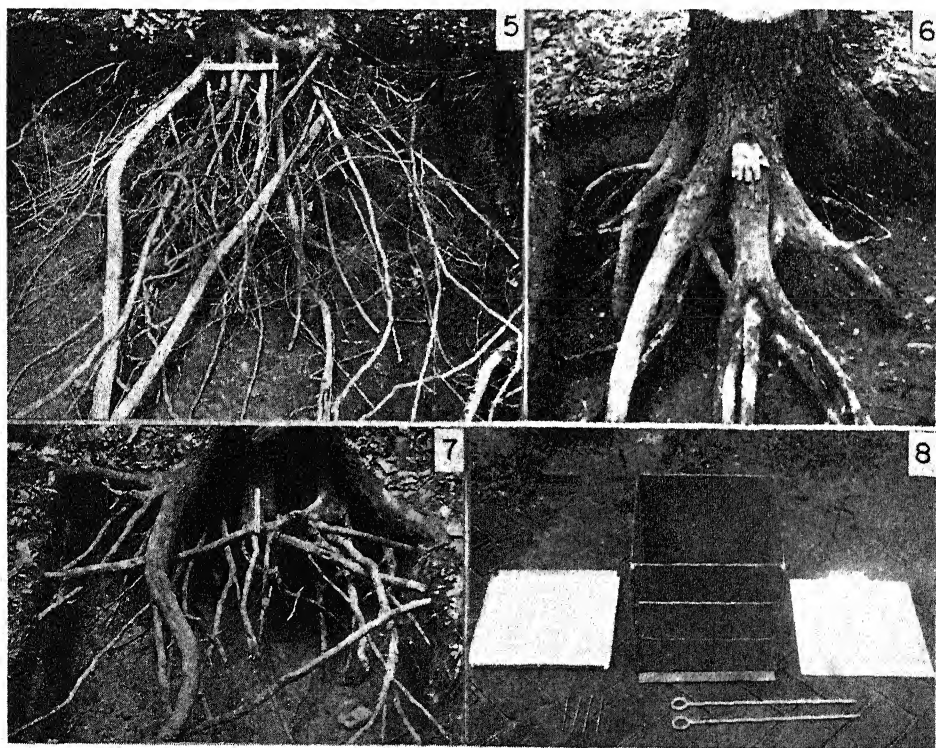
The methods used at stations 1-10 were, with some variations, similar to those initially employed by WEAVER (18) and those recently followed by TURNER (16),



FIGS. 1-4.—General views of some station types. Fig. 1, station 9; fig. 2, station 13; fig. 3, station 4; fig. 4, station 2.

BILLINGS (2), LUTZ, ELY, and LITTLE (10), and COILE (3). The rectangular trenches employed were $7\frac{1}{2} \times 2\frac{1}{2}$ feet. The area charted was 6 feet long horizontally, to the depth of the trench vertically, and on the wall nearest magnetic north. Stations 11-16 were sampled by a new method in which equal areas of all four walls of a square trench were charted. Such trenches were 4 feet square. The area charted on each wall was 3 feet long and to the depth of the trench (fig. 21).

After a station was chosen, either a rectangular or square trench was opened in a representative area sufficiently removed from trunks of any large trees and to a depth in the C horizon averaging $3-3\frac{1}{2}$ feet, where the roots were significantly few in number. Vertical walls were maintained and roots projecting into the trench were cut off. After the wall or walls to be charted had been cleaned, the horizontal

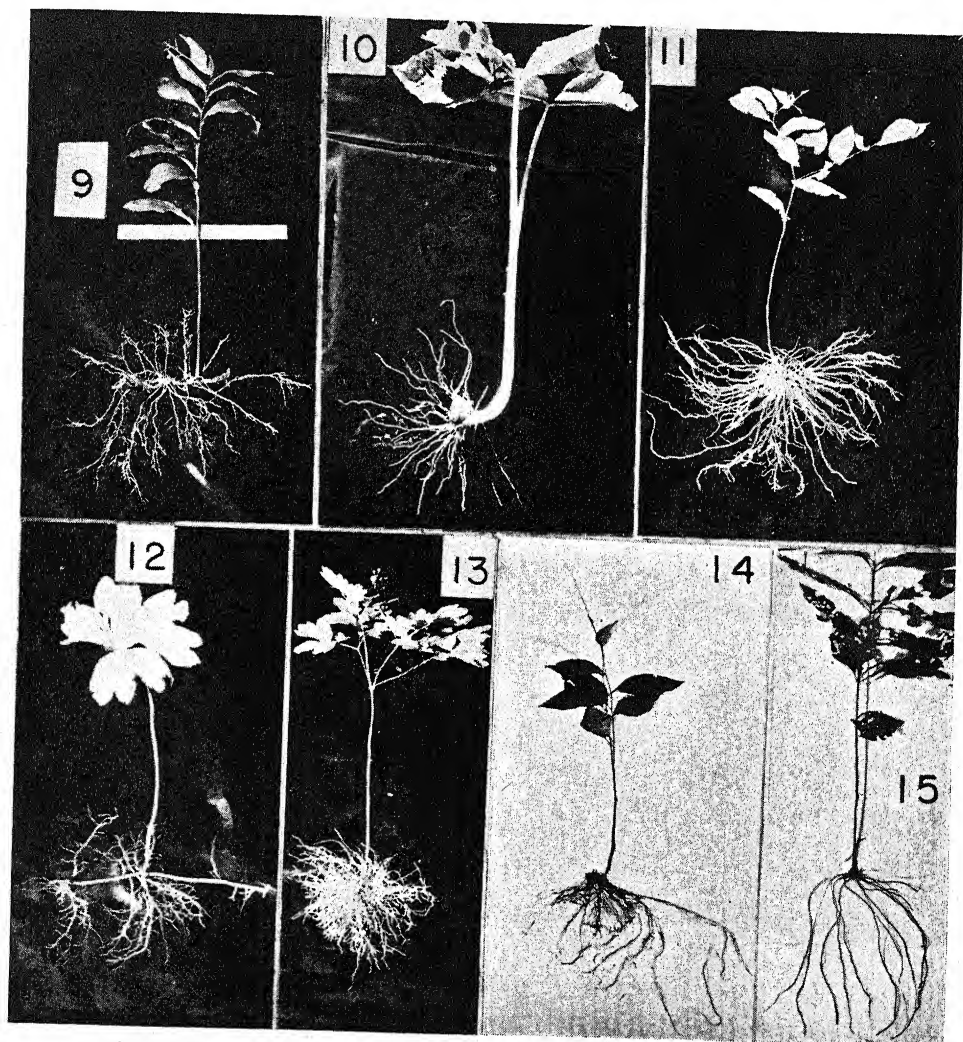


FIGS. 5-8.—Figs. 5-7, excavated specimens of dominant tree species: 5, *Acer saccharum*; 6, *Quercus alba*; 7, *Quercus borealis maxima*. Fig. 8, soil sample box for root-volume determinations.

boundaries of the profile were marked. Each area was ruled into square feet by horizontal and vertical strings. The horizons were charted on a scale of 3 inches to 1 foot.

A heavy, square-foot wire frame, divided into 2-inch squares by fine wire, was used in charting all profile inclusions. This was placed successively on each square foot of the trench face, which was carefully worked over with a fine pointed instrument to locate all root ends. Living, dead, woody, and herbaceous roots were then charted. Seven diameter size classes were recognized: 0-1, 1-2, 2-3, 3-4, 4-5, 5-10, and > 10 mm.

After charting the roots, the horizons were examined for other characteristics and possible inclusions. Counts were made of earthworms and cicada nymphs,

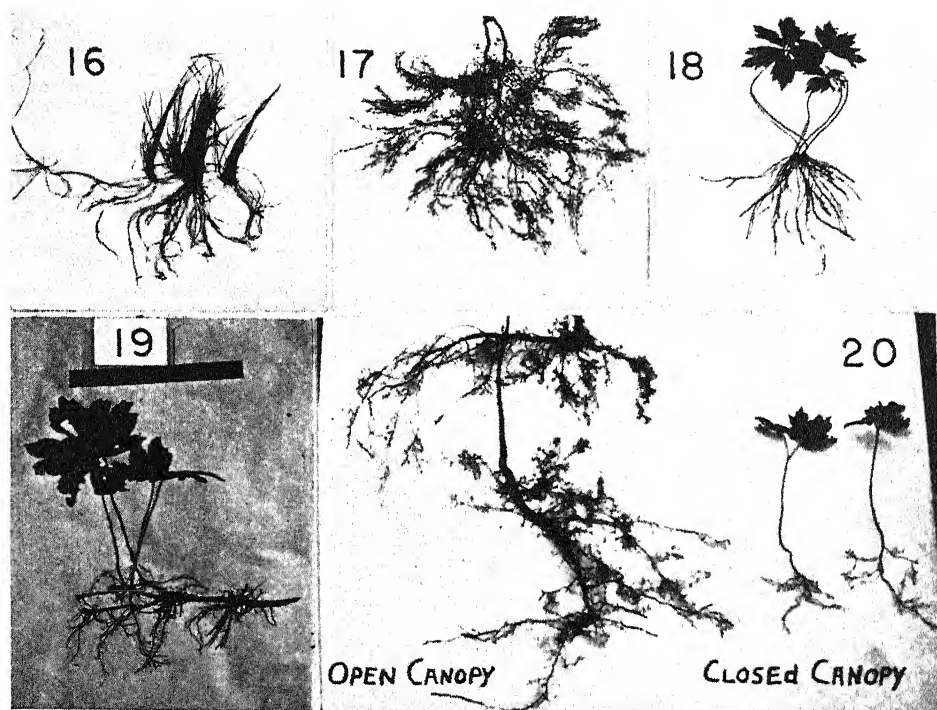


FIGS. 9-15.—Root systems of some native species. Fig. 9, *Smilacina racemosa*; fig. 10, *Arisaema triphyllum*; fig. 11, *Uvularia grandiflora*; fig. 12, *Podophyllum peltatum*; fig. 13, *Caulophyllum thalictroides*; fig. 14, *Phryma leptostachya*; fig. 15, *Eupatorium purpureum*.

and their burrows were recorded on the profile chart. The root systems of characteristic woody seedlings and herbaceous species, located either at or near the edge of a trench, were excavated and their relations to the surface horizons

noted. In other cases the soil was washed away from excavated blocks. The entire plants, or occasionally only their root systems, were photographed (figs. 9-20).

Root volumes were determined in the upper cubic foot of soil in different station types. For this purpose a soil-sample box was devised, permitting the subdivision



FIGS. 16-20.—Root systems of some native species. Fig. 16, *Carex* sp.; fig. 17, *Solanum dulcamara*; fig. 18, *Geranium maculatum*; fig. 19, *Parthenocissus quinquefolia*; fig. 20, *Acer saccharum* (1936 seedlings from open and closed canopies).

of a sample into three successive sections, each 4 inches in depth. The box was constructed of $\frac{1}{8}$ -inch rolled-sheet steel welded to give an inside volume of 1 cubic foot. The bottom edges were beveled to make a sharp cutting edge. The plate used to divide the total sample into three units, 4 inches deep, was driven, guided by two slightly raised lips, through two narrow slots on one side of the box. By successive driving and digging around the box, an accurate cubic-foot sample, with its roots, could be obtained (fig. 8). Roots $1\frac{1}{2}$ -2 inches in diameter were easily cut in preliminary sampling. Root volumes were measured by displacement in water.

STATISTICAL METHODS

In measuring the degree of heterogeneity exhibited by the root distribution in the various station types, it was necessary to treat the data statistically. The

standard deviation of means, that is, standard error (standard error $\sqrt{\frac{\sum V^2 - \frac{(\sum V)^2}{N}}{N(N-1)}}$,

where \sum is the symbol for summation, V is the individual observation, and N is the number of variants) was obtained for each wall of square trenches as well as for total root data of the four walls.² Accordingly, the limits within which variations of the means occur because of uncontrollable conditions were defined. The standard error of the difference (standard error of difference = $(S.E._x)^2 + (S.E._y)^2$, where $S.E._x$ and $S.E._y$ are the standard errors of the means of x and y , respectively, was determined when two walls of the same square trench were compared. The

t test for significance was used accordingly ($t = \frac{\bar{D}}{S.E._{\bar{D}}}$, where \bar{D} is the difference between means and $S.E._{\bar{D}}$ is the standard error of the difference). The value t was used to determine the probability (P) according to the table for P values as given by FISHER (5). Differences not significant at the 0.05 level were not considered statistically significant (14).

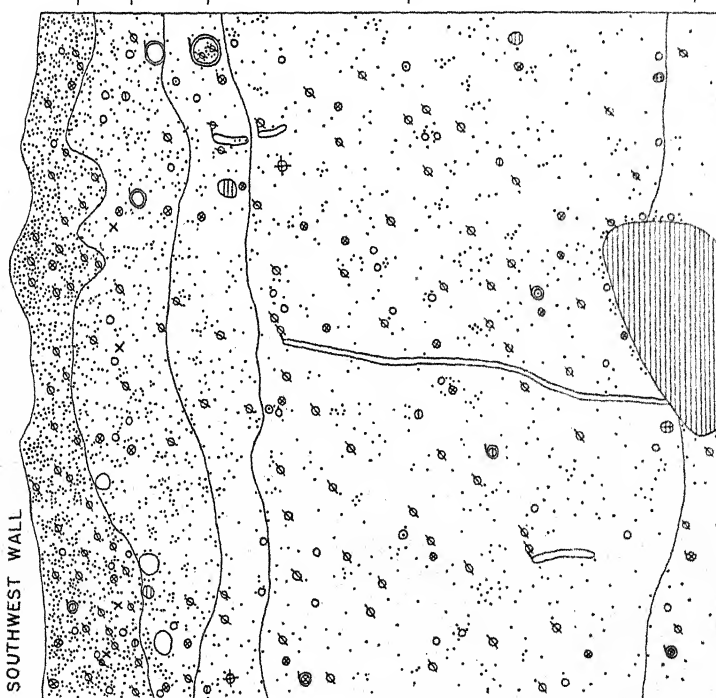
SOIL FACTORS

The soil surface about station types with sparse ground cover (stations 1, 2, 3, 14, and 16) showed varying degrees of erosion, evidenced by the partial exposure of tree roots 2–3 inches in diameter and the occasional appearance of the A_2 and B_1 horizons. Judged by its typical grayish yellow color, the A_2 horizon was absent in portions of the profile sections in stations 7 and 9. In stations 4, 5, and 11, with ground cover predominately of sedge, all horizons were characterized by greater sandiness than at other stations.

In all profiles, the B_2 horizons (of heavy, reddish brown sandy clay) possessed the greatest depth and compactness. It typically showed many roots penetrating along the sutures formed by the irregular nut structure. The smallest roots (0.1–1.0 mm.) frequently were found penetrating the smaller soil blocks in the upper portions of the horizon, while this condition was relatively rare in the lower, more compact soil with larger blocks. Resistance to root penetration was evident from the flattened form of the smaller roots (< 2 mm.), when following the suture lines. Internal drainage is greatest along the sutures of the nut structure. It appeared comparatively favorable except in stations 1, 2, 3, 14, and 16, where greater

² Square trenches readily permit application of statistical methods. Accordingly, statistical treatment was confined to comparisons of variability in root numbers among station types sampled by such trenches and among their four walls (table 7).

SOUTHWEST WALL



NORTHWEST WALL



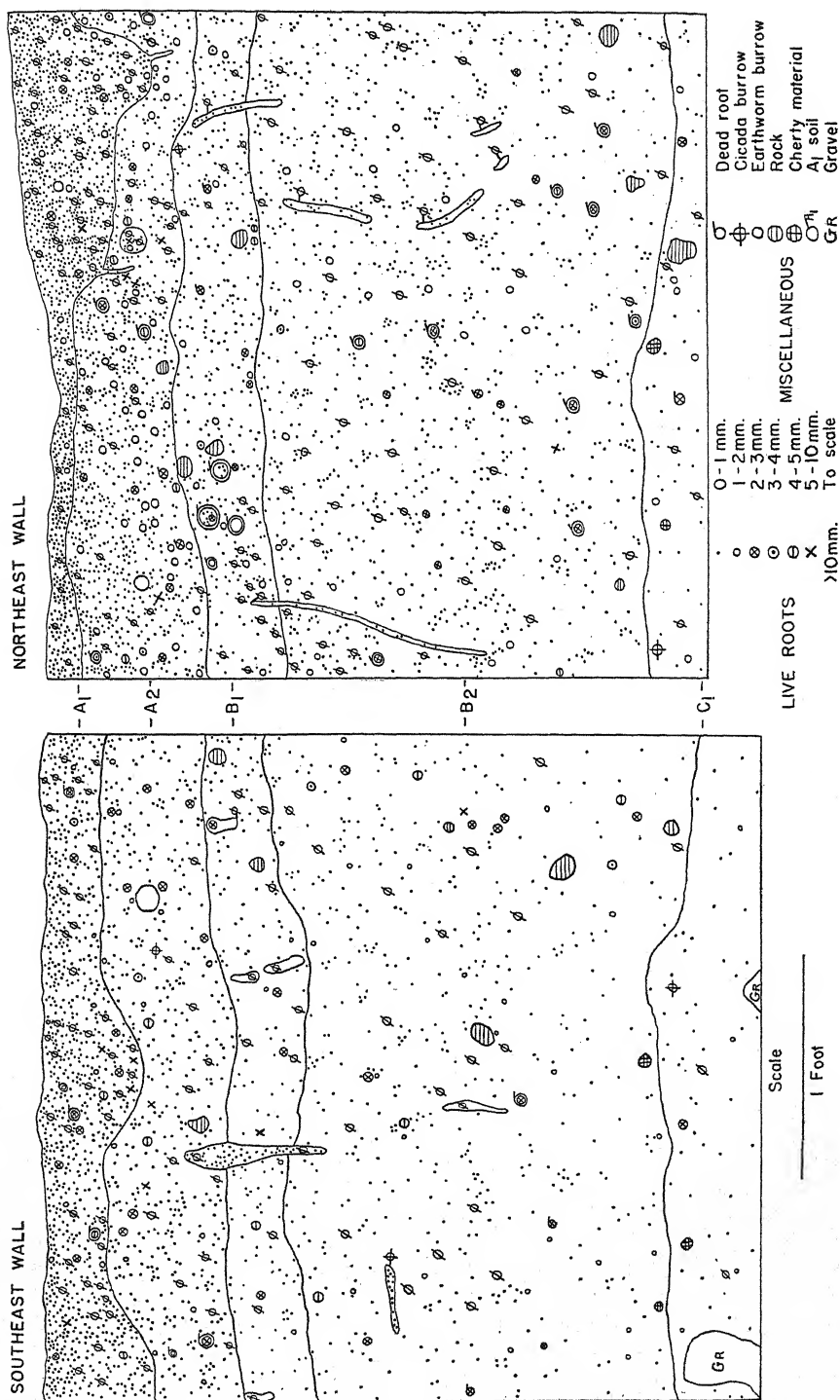


FIG. 21.—Root distribution and other horizontal inclusions in S.W., N.W., S.E., and N.E. profile walls of station 12; 1939

plasticity of the B₂, with marked increase in the clay content, interferes with percolation. Conversely, conditions for greatest drainage were found in stations 4, 5, and 11, with distinctly sandier profiles.

The surface soil of all stations exhibited numerous earthworm (*Lumbricus* spp.) middens (6) of variable diameter and height. Typically they were 4-5 inches wide and 1-1½ inches high. Cross-sections of some of the middens and accompanying burrows have shown leaf blades and petioles, in various stages of decomposition, to a depth of 6-8 inches. Burrows of smaller earthworms were also found. The larger burrows were lined with a film of dark organic soil which originated from worm castings; the burrows of the smaller earthworms had no visible organic soil lining. Apparently lining of burrows is accomplished over several years of active use. Middens play an important part in giving the surface soil an irregular local topography. Their black, highly organic soil withstands erosion very effectively. Some of the height of those located on slopes was developed by erosion about them.

Earthworms are the most conspicuous group of animals appearing in the profile sections. Greatest activity was manifest in the A₁ horizon, where they are largely responsible for the soil's porous nature. Numerous castings studded the middens proper and the soil surface at some distance from them. Burrows were found also throughout midden-less areas. The worms apparently play as important a role in mixing and aerating the surface horizons (A₁ and A₂) as they do in breaking down the leaf litter. In the latter case it is known that earthworms do eat leaf materials (4), yet their over-all effect upon breakdown of leaf litter probably centers about creation of a more favorable environment for bacteria and fungi through movement of leaves into the burrows.

In all profiles, regardless of the resistant nature of the B horizons, earthworms and their burrows were found in the C horizon at depths of 3-3½ feet, and in deeper trenches at 5 feet. The burrows were fairly perpendicular, with deviations largely where rocks blocked the path. No differences in depth of penetration were found in the profiles investigated.

The periodical cicada, *Magicicada septendecim* (15), was also conspicuous. The stage initially encountered in 1938 was the fifth instar or last stage prior to the adult. The former emerged during the spring of 1939. The varied nymph and pupal stages (1, 12) of the insect had penetrated the profile to depths of 3-3½ feet. In 1938 some nymphs were found in pockets which, approximating ½-inch in diameter and ¾-1 inch in height, just housed them. More typically this chamber extended upward several inches, a nymph lying at the bottom. In the latter cases the nymphs apparently had started their upward movement through the soil, but such burrows ended abruptly in either the B₂ or C₁ horizon. They were never found in the upper horizons (A₁, A₂, or B₁).

Burrows, formed by nymphs in the previous generation, had apparently been filled.³ The trenches opened in 1939 revealed the burrows resulting from emergence of the nymphs. Mounds, built by the nymphs over the mouths of their burrows, were composed of B horizon soil, had an average basal diameter of 1 inch and a height of $1\frac{1}{2}$ – $2\frac{1}{2}$ inches, with walls $\frac{3}{16}$ – $\frac{4}{16}$ inch thick. They typically were confined to wooded areas where organic forest debris was abundant on the surface soil. In open areas outside the forest such mounds were rare.

Open cicada burrows, extending almost perpendicularly from their surface openings to varying depths, were common in all profiles investigated in 1939 and 1940. On 30-meter quadrats sampled at random, an average of fourteen cicada mounds per square meter was found.

In the A₂, B₁, and B₂ horizons of all profiles occurred varying-sized pockets of A₁ soil, from the filling-in of old root holes after root decay. Similar smaller pockets were apparently the result of earthworm activity, particularly in the A₂. The castings were conclusive evidence in differentiating these latter pockets from filled-in root holes, which also often contained fragmentary and undecomposed portions of roots. Some A₁ soil probably washes into earthworm burrows. Small herbaceous and woody roots often were found where, as a result of the union of several adjacent burrows, pockets of dark soil were developed with diameters of $\frac{1}{2}$ inch or more. This was also true in root holes which had been filled to the lower levels of the B₂ horizon. The concentration of roots in such pockets frequently approached, and occasionally equaled, that typical of the A₁ horizon. The roots in such pockets did not have the flattened form typical in the B₂. Some pockets of apparently A₁ soil in the B horizons, approximating $\frac{1}{2}$ inch in diameter, were probably the filled-in burrows of the cicada nymphs which emerged in 1922. Still larger pockets (6–8 inches in diameter) probably were filled-in burrows of rodents.

ROOT DATA

The basic data on root distribution (table 2) indicate the total number of live roots in the horizons of each station. The seven size classes recognized are differentiated into two groups, < 1 mm. and > 1 mm. The number of roots per square foot of horizon surface was greatest in horizons A and B, the former always with a much larger number than the latter. The number of roots per square foot of C horizon was low in all cases. The apparent relative suitability of horizons A₁, A₂, B₁, B₂, and C₁ for root development, as measured by numbers, decreased—with a few exceptions—in passing downward. With the exception of stations 5, 7, and 15, the greatest total decrease in root numbers per square foot of horizon surface occurred in passing from the A₁ to the A₂, although the latter was encountered

³ Mr. WILLIAM P. LONGLAND, superintendent of Wychwood, stated that cicada nymphs had emerged in exceedingly large numbers in 1922. He judged the numbers in 1939 to be equally as great.

at the relatively slight average depth of 4 inches. These three stations showed marked exception to the typical root distribution.

TABLE 2
NUMBER OF LIVE ROOTS PER PROFILE HORIZON

HORIZON	TOTAL NO. OF ROOTS	No. of ROOTS PER SQUARE FOOT OF ROCK-FREE AREA		TOTAL NO. OF ROOTS	No. of ROOTS PER SQUARE FOOT OF ROCK-FREE AREA		TOTAL NO. OF ROOTS	No. of ROOTS PER SQUARE FOOT OF ROCK-FREE AREA		TOTAL NO. OF ROOTS	No. of ROOTS PER SQUARE FOOT OF ROCK-FREE AREA	
		<1 MM.	>1 MM.		<1 MM.	>1 MM.		<1 MM.	>1 MM.		<1 MM.	>1 MM.
A ₁ A ₂ B ₁ B ₂ C ₁	STATION 1*			STATION 2			STATION 3			STATION 4		
	460	116	31	591	450	23	1067	423	55	2072	1028	41
	230	33	14	273	87	20	311	166	22	684	218	25
	50	12	3	111	46	11	523	91	15	453	89	9
	99	8	2	380	27	4	137	46	5	304	60	6
	14	5	13	7	2	166	33	1	403	55	6
	STATION 5			STATION 6			STATION 7			STATION 8		
	1857	1289	6	2487	657	30	1185	526	16	1781	870	21
	1495	740	71	436	222	11	451	649	20	797	213	11
	315	130	14	383	138	8	785	401	23	375	172	13
B ₂	965	68	6	644	116	7	1537	157	9	772	66	6
C ₁	11	9	2	270	70	1	18	68	65	32	3
A ₁ A ₂ B ₁ B ₂ C ₁	STATION 9			STATION 10			STATION 11			STATION 12		
	1912	680	31	1873	868	22	3541	891	31	3897	1094	24
	256	381	29	1060	353	21	2268	371	22	2510	404	28
	405	195	11	379	225	18	862	216	10	927	252	12
	1070	98	7	1074	93	7	3021	133	8	3009	136	8
	180	107	8	181	91	3	431	57	4	220	65	7
	STATION 13			STATION 14			STATION 15			STATION 16		
	4656	820	50	2043	554	28	1595	354	41	1666	453	57
	1763	321	26	1281	207	15	1081	378	53	1004	129	18
	721	211	11	438	177	11	362	161	17	416	114	16
B ₂	1707	113	10	1695	87	9	1443	59	7	852	55	13
C ₁	456	42	5	410	49	4	206	37	4	314	22	7

* Data for stations 1-10 based on one wall 6 feet long; for stations 11-16, on four walls totaling 12 feet long.

A concentration of the smallest roots, 0.1-1.0 mm. in diameter, was found in the A₁ horizon (table 2), indicating the location of much of the root water-absorbing

surface. This size class, per unit area, decreased markedly in the lower horizons. Contrary to possible expectations, the larger roots (> 1 mm.) showed no increase in numbers per unit horizon surface below the A. Further, within the A horizon only stations 5, 7, and 15 showed such an increase in the A_2 as compared with the

TABLE 3
ROOT NUMBERS AND PERCENTAGE ROOT AREA PER SQUARE FOOT OF
ROCK-FREE HORIZON SURFACE*

STATION	ROOT UNIT	HORIZON				
		A_1	A_2	B_1	B_2	C_1
1	No.....	147	47	15	10	5
	Area.....	1.913	0.341	0.025	0.029	0.001
2	No.....	473	107	57	31	9
	Area.....	0.535	0.365	0.399	0.122	0.052
3	No.....	478	188	106	51	34
	Area.....	1.291	0.217	0.119	0.012	0.004
4	No.....	1069	243	98	75	61
	Area.....	0.676	0.276	0.162	0.025	0.096
5	No.....	1295	811	144	74	11
	Area.....	0.441	1.459	0.243	0.031	0.006
6	No.....	687	233	146	123	71
	Area.....	1.817	0.157	0.321	0.248	0.017
7	No.....	542	678	484	166	68
	Area.....	0.275	0.407	0.584	1.023	0.001
8	No.....	891	224	185	72	35
	Area.....	0.360	0.204	1.363	0.581	0.015
9	No.....	711	410	205	105	115
	Area.....	0.813	0.138	1.360	0.447	0.108
10	No.....	890	374	243	100	94
	Area.....	0.987	0.423	0.217	2.115	0.031
11	No.....	922	293	226	141	61
	Area.....	0.723	0.555	0.195	0.121	0.064
12	No.....	1118	492	264	144	72
	Area.....	0.758	1.078	0.301	0.099	0.075
13	No.....	870	347	222	123	47
	Area.....	2.116	0.642	0.180	0.176	0.111
14	No.....	582	272	188	96	53
	Area.....	0.948	0.391	0.218	0.093	0.064
15	No.....	395	431	178	66	41
	Area.....	0.416	1.459	0.309	0.093	0.025
16	No.....	510	147	130	68	29
	Area.....	0.647	0.221	0.259	0.240	0.257

* Here and in subsequent tables, profile data for stations 1-10 based on one wall 6 feet long; for stations 11-16, on four walls totaling 12 feet long.

A_1 . Greatest numbers of dead roots were found in the lower horizons. The smaller roots (< 5 mm.) were found dead more frequently.

From the root distribution recorded on the charts in the field, the number of living roots and the percentage cross-sectional area occupied by them were calculated on three different bases for each station. These were (a) root numbers and percentage area per square foot of rock-free horizon surface (table 3); (b) root

numbers and percentage area per square foot of rock-free vertical profile sections occupying 0-1, 1-2, 2-3 foot depths (table 4); and (c) root numbers and percentage area per square foot of vertical profile sections 3 feet in depth (table 4). Root areas were obtained by use of the seven diameter size classes. Roots 0.1-1.0

TABLE 4
ROOT NUMBERS AND PERCENTAGE ROOT AREA PER SQUARE FOOT OF ROCK-FREE
VERTICAL PROFILE SECTIONS 0-1, 1-2, 2-3, AND 0-3 FEET DEEP

STATION	DEPTH (FEET)	ROOT NO.	ROOT AREA (%)	STATION	DEPTH (FEET)	ROOT NO.	ROOT AREA (%)
1	0-1.....	96	1.191	9	0-1.....	444	0.361
	1-2.....	15	0.047		1-2.....	105	0.059
	2-3.....	10	0.029		2-3.....	79	0.035
	0-3.....	41	0.422		0-3.....	209	0.152
2	0-1.....	163	0.438	10	0-1.....	525	0.255
	1-2.....	41	0.139		1-2.....	130	0.199
	2-3.....	23	0.065		2-3.....	123	0.178
	0-3.....	76	0.214		0-3.....	259	0.211
3	0-1.....	275	0.606	11	0-1.....	528	0.547
	1-2.....	60	0.030		1-2.....	179	0.157
	2-3.....	25	0.003		2-3.....	96	0.114
	0-3.....	120	0.214		0-3.....	268	0.273
4	0-1.....	477	0.386	12	0-1.....	603	0.767
	1-2.....	88	0.097		1-2.....	174	0.167
	2-3.....	82	0.094		2-3.....	95	0.084
	0-3.....	216	0.192		0-3.....	291	0.339
5	0-1.....	607	0.865	13	0-1.....	573	1.249
	1-2.....	99	0.032		1-2.....	137	0.113
	2-3.....	48	0.028		2-3.....	65	0.101
	0-3.....	251	0.308		0-3.....	258	0.488
6	0-1.....	501	0.359	14	0-1.....	330	0.501
	1-2.....	135	0.067		1-2.....	98	0.238
	2-3.....	84	0.005		2-3.....	55	0.112
	0-3.....	240	0.143		0-3.....	161	0.284
7	0-1.....	486	0.305	15	0-1.....	284	0.572
	1-2.....	143	0.064		1-2.....	62	0.081
	2-3.....	55	0.012		2-3.....	40	0.041
	0-3.....	228	0.127		0-3.....	129	0.231
8	0-1.....	467	0.213	16	0-1.....	233	0.307
	1-2.....	113	0.208		1-2.....	76	0.213
	2-3.....	48	0.024		2-3.....	34	0.342
	0-3.....	210	0.148		0-3.....	114	0.287

mm. were assigned diameters of 0.75 mm. Exact diameters of roots > 10 mm. were obtained in the field. Accepted diameters of the five remaining size classes were the midpoints of their ranges.

Based on root numbers and percentage root area per square foot, the suitability for root growth of the specific horizons A₁, A₂, B₁, B₂, and C₁ was variable. Twelve stations showed both greater root numbers and higher percentage root area in the A₁ than in the A₂. Of the other stations, only two—stations 7 and 15—showed both greater root numbers and area in the A₂ than in the A₁. The latter stations were

selected because a dense and homogeneous layer of *Parthenocissus quinquefolia* dominated the ground cover of both areas. The roots of this species are found almost exclusively in the upper portions of the A_2 horizon. In station 5 there was a higher percentage of root area in the A_2 horizon but not a greater number of roots. Here the difference coincided with the association of a large number of small shrubs with comparatively homogeneous patches of sedge. In no station did the B_1 horizon show both greater root numbers and percentage root area than did the A_2 . This was true successively in the B_2 and C_1 , where the influence of large roots upon the calculated area gave higher values that were not significant. In terms of both root area and numbers, however, the B_2 and C_1 horizons exhibited the least suitability for root development and showed less variation from the one to the other than did the upper horizons. These general statements, with the few exceptions, hold true regardless of the peculiarities of the different stations.

The greater total and percentage decreases of both root number and area, in profile sections 0-1, 1-2, and 2-3 feet in depth (table 4), occurred in passing into the second foot. The decrease was less from the second to the third foot. Station 8 decreased more in area in passing from the second to the third foot but showed typical decreases in root numbers with increased depth. The greatest root area in station 16 occurred in the third-foot section. These two stations, however, showed greater total and percentage decrease of root numbers from the first to the second foot level. Generally, the larger the root numbers and total area in the top sections, the greater the percentage decrease at the second foot level. Root frequency definitely decreases with increased depth.

Profile areas 3 feet in depth (table 4) at stations 1, 2, 3, 14, and 16, selected because of dearth of ground cover, were characterized by comparatively large percentage root areas and small root numbers, that is, few but large roots. Stations 4-13 and 15, with abundant ground cover of varied floristic composition, were characterized by large root numbers and small average root areas (table 2). Station 15, an exception to this general condition, had an average area per root approximately equal to that of station 14, which possessed the smallest average area per root of the five "bare" stations. This can be explained in part by the association of a number of woody species (table 1) with the society of *Parthenocissus* sampled at the former station, influencing the number of roots > 1 mm.

The number of earthworm burrows per square foot of profile areas indicated great activity in all station types. Such burrows occurred less frequently in stations where patches of sedge were associated with sandier silt loam soils. The greatest number of cicada pupae and burrows were found where red oaks were dominant in the canopy, but the nymphs and their burrows were found in all the trenches (table 6). Such burrows afforded significant drainage during rains heavy enough to yield run-off.

The total volume of roots in an upper cubic foot of soil was variable throughout the station types (table 5). Areas with sparse ground cover yielded root volumes equal to those of areas with abundant ground cover. In samples which contained no roots $> \frac{1}{2}$ inch in diameter, root volume decreased successively in the 0-4, 4-8,

TABLE 5
VOLUME OF ROOTS IN ONE CUBIC-FOOT SOIL BLOCKS
SUBDIVIDED INTO 4×12×12 INCH SECTIONS

SAMPLE	GROUND COVER TYPE	DEPTH OF SOIL BLOCK SECTION (INCHES)	PERCENTAGE ROOT VOLUME	
			PER SOIL BLOCK SECTION (4×12×12 INCH)	IN TOTAL CUBIC FOOT
1	Mixed mesic spp.	0-4.....	0.481	0.261
		4-8.....	0.167	
		8-12.....	0.136	
2	Mixed mesic spp.	0-4.....	0.659	0.453
		4-8.....	0.628	
		8-12.....	0.073	
3	Bare	0-4.....	0.963	2.164
		4-8.....	4.272	
		8-12.....	1.256	
4	Bare	0-4.....	0.235	0.535
		4-8.....	1.301	
		8-12.....	0.010	
5	C. pennsylvanica society	0-4.....	1.717	1.148
		4-8.....	1.549	
		8-12.....	0.178	
6	C. pennsylvanica society.....	0-4.....	2.795	2.494
		4-8.....	4.550	
		8-12.....	0.136	
7	Lonicera dioica society	0-4.....	1.413	0.530
		4-8.....	0.157	
		8-12.....	0.020	
8	Lonicera dioica society	0-4.....	1.895	0.813
		4-8.....	0.471	
		8-12.....	0.073	
9	Parthenocissus sp. society	0-4.....	0.701	0.656
		4-8.....	1.151	
		8-12.....	0.115	
10	Parthenocissus sp. society	0-4.....	1.266	0.484
		4-8.....	0.104	
		8-12.....	0.083	

and 8-12 inch levels. When the sample contained larger roots, the volume frequently was largest in the 4-8 inch level; but the 8-12 inch section never surpassed the 4-8 inch level. In all samples the 0-8 inch section contained 80 per cent or more of the total root volume.

The root systems of typical herbaceous and woody seedlings predominately

were confined to the upper 6 inches of the profile (figs. 9-20). Exceptions occurred in areas where openings in the canopy have existed for several years. There most root systems extended to depths of 12-18 inches.

The root systems of the selected specimens of the three dominant species of the forest—sugar maple, red oak, and white oak—were investigated in 1940 (figs. 5-7). The white oak, 223 years old, 23 inches in diameter at a height of 2 feet, was wind-

TABLE 6
COMPARATIVE STATION TYPE DATA

STATION	PHYSIOGRAPHY		DEPTH TO C. HORIZON (INCHES)	IMPORTANT SPECIES OF GROUND VEGETATION	NO. OF SPECIES WITH 50-100% FREQUENCY INDEX		EARTH-WORM BURROWS PER SQUARE FOOT	CICADA BURROWS PER TOTAL PROFILE
	CHARACTER OF TOPOGRAPHY	ELEVATION (FEET)			HERBACEOUS (11 SPECIES RECORDED)	WOODY (10 SPECIES RECORDED)		
1...	Southeast slope, 2.5°	961	36-42	Mesic woodland herbs and shrubs (rare)	4	5	14	3
5...	South slope, 4.5°	1019	36-43	<i>Carex pennsylvanica</i> (abundant)	6	8	8	3
7...	Southeast slope, 7.5°	1017	32-38	<i>Parthenocissus quinquefolia</i> (abundant)	7	7	7	6
8...	South slope, 4.5°	909	36-40	Mesic woodland herbs and shrubs (abundant)	9	6	9	3
11...	North slope, 3.5°	1011	29-36	<i>C. pennsylvanica</i> (abundant)	9	8	9	15
12...	Northwest slope, 2.5°	961	33-40	Mesic woodland herbs and shrubs (abundant)	9	8	17	9
13...	Northwest slope, 13°	1009	25-30	<i>Lonicera dioica</i> (abundant)	7	10	12	10
14...	Northeast slope, 4.5°	998	28-33	Mesic woodland herbs and shrubs (rare)	7	7	9	10
15...	South slope, 2.5°	972	29-35	<i>P. quinquefolia</i> (abundant)	10	10	21	4
16...	Southeast slope, 7°	951	30-35	Mesic woodland herbs and shrubs (rare)	8	9	14	4

broken in 1938. Its root system was the most massive of the three species. Typically deep rooted, it proportionately contributed a smaller amount of root area in the upper A₁, A₂, and B₁ than did the specimens of red oak and sugar maple studied. The red oak partially excavated was 97 years old, 22 inches in diameter at a height of 2 feet, and was also wind-broken in 1938. Its root system was less massive than that of the white oak but contributed heavily in both large and small roots to the upper three horizons. No large sugar maple was available for excavation, but a smaller specimen, wind-broken in 1940, was investigated. It consisted of two

sprouts, each 15 years old, and had a diameter of 4 and $3\frac{1}{2}$ inches, respectively, at a height of 2 feet. The most marked character of the root system was the mass of smaller roots ($< \frac{1}{4}$ inch in diameter) which ramified throughout the upper three horizons (fig. 5). The relative number of deep anchorage roots was as great as in the red and white oaks. The response of maple seedlings to the effects of increased insolation following tree wind-fall or wind-break and subsequent canopy opening was also of interest (fig. 20).

Discussion

The significance of some of the findings has been treated in sections on soil factors and root data. On the whole, the results are in accord with those of previous investigators. COILE (3) examined four profiles in an uneven-aged stand of white oak-black oak-red oak on Georgeville stony clay in the Duke Forest, North Carolina. KORSTIAN and COILE (9), in reporting the same study, state: "The most striking features are the relatively large number of small roots in the upper few inches of soil and the paucity of roots of the two smallest size classes (< 0.1 and 0.11 to 0.30 inch) in the B and C horizons." While this condition is also true at Wychwood for comparable size classes (table 2), the present profiles, regardless of type of trench employed, showed greater numbers of roots in all horizons than were encountered by COILE. The only profiles comparable with his in number of roots per horizontal square foot were those here investigated because of scarcity of herbaceous and woody ground cover (stations 1, 2, 3, 14, and 16). The differences in root numbers probably are due to the regional locations of the respective stands and to differences in soil types and amount of herbaceous ground cover.

The soil type at Wychwood, Bellefontaine silt loam, obviously is capable of allowing the development of comparatively large numbers of roots, particularly in the A_1 and A_2 horizons. In the B_2 horizon the roots were found to penetrate the soil in comparatively large numbers, not only along the sutures of the characteristic nut structure, but also—though less frequently—through the nutlets.

In accordance with earlier findings (16, 2, 10, 3, 13, and others), this study has shown a differential suitability for root development in the different horizons. The greater number and percentage cross-sectional root area per square foot in the A_1 horizon was expected, for it is here that oxygen, water, and nutrient supplies are usually most favorable. The exception in stations 7 and 15, in which the A_2 surpasses the A_1 in numbers and root area, indicates that regardless of the potential suitability of a horizon for root development, the actual root distribution is conditioned by the type of root system of the dominant or subdominant species in the area. Further, root systems of such ground cover species as *Parthenocissus quinquefolia* will distort the results in any study where an attempt is made to measure only tree roots, unless great care is taken to identify each woody root encountered, particularly smaller ones. Especially is this true in deciduous forests, where

herb and shrub ground cover makes it almost impossible to obtain an accurate measure of tree roots only.

The concentration of root numbers and cross-sectional root area in the upper foot of the profiles suggests that aeration is possibly of significance. This point was further emphasized by the low number of smaller roots in lower levels of the profiles. Correspondingly there were more dead roots in the lower depths. While such findings point to lack of aeration as a limiting factor for root development with increased depth, it is probably not the only factor involved. This view is substantiated in areas where the canopy has been opened through wind-break or wind-fall, where tree seedling and herbaceous root development is greater at all levels of the profile. Thus it is debatable whether the greater development of roots in the upper part of the soil is a response to more favorable aeration, moisture, or temperature conditions. These factors, as well as a more abundant supply of available nutrients and inherent tendencies of native species when grown under low light intensity, probably are interrelated in influencing the greater root development in the upper soil section.

Areas comparatively free of ground vegetation—for example, stations 1, 2, 3, 14, and 16—had fewer roots although they possessed a higher average cross-sectional area per root than did those stations with abundant ground cover, regardless of type. The findings in the former stations thus primarily indicate the root condition of the trees which make up the canopy. On the other hand, root numbers recorded for profiles of “bare” stations are in themselves comparatively large. An area with abundant ground cover was found (station 15) whose root numbers and area equaled that of a “bare” area. This suggests that the relative proportion of woody to herbaceous roots fluctuates throughout the forest. It is possible that the woody roots in bare areas may draw heavily upon the supplies of the soil environment and thereby limit the establishment of herbaceous species. Unpublished results of trenched quadrat studies, however, suggest that such root competition is not critical.

When stations 1-10 were sampled during the summer of 1938 by rectangular trenches, it seemed that the selected wall was not always typical of the root distribution on the four walls. This was particularly true in areas with abundant ground cover where several herbaceous or woody plants appeared near one or more of the walls. Because of this visible local heterogeneity in root distribution, it was decided to test the usefulness of square trenches—in which equal areas on all four walls are charted. Such sampling should diminish the influence of root systems which, with predominating numbers, approach the trench from one direction. Further, since the problem of sampling root distribution is basically statistical, the method should yield a more accurate measure of the distribution with respect to all directions, especially where undue directional effects are evident. In the square trenches the root data from the four walls may be totaled.

In table 7, the two walls of each trench varying the most with respect to the A_1 horizon are compared statistically as to root numbers. Results much more significant were obtained when horizon extremes of each trench were compared. However, since only one wall would have been selected in using a rectangular trench, the comparisons are on a wall-for-wall basis.

The probability of occurrence for differences between two walls of the same trench ranged from 1 chance in 100 (very significant) to 1 chance in 2 (no significance). In station 12, located on a 2.5° slope, the two walls showed significant differences in the upper four horizons. Stations 11, 13, 14, 15, and 16 had walls

TABLE 7

t TEST OF ROOT NUMBER MEAN DIFFERENCES BETWEEN WALLS OF SQUARE TRENCHES

STATION	WALLS	HORIZON	$\bar{D}/S.E._D$	VALUE OF t	STATION	WALLS	HORIZON	$\bar{D}/S.E._D$	VALUE OF t
11	N.W. vs. N.E.	A_1	517/157	3.292*	14	E. vs. W.	A_1	200/60	3.028*
		A_2	136/42	3.238*			A_2	44/13	3.384*
		B_1	45/36	1.250			B_1	64/36	1.777
		B_2	5/12	0.416			B_2	13/5	2.600
		C_1	24/8	3.000*			C_1	8/6	1.333
12	S.E. vs. N.E.	A_1	564/93	6.064†	15	E. vs. S.	A_1	126/45	2.800*
		A_2	337/85	3.964*			A_2	166/109	1.495
		B_1	122/15	8.133†			B_1	84/28	3.000*
		B_2	85/15	5.666*			B_2	3/4	0.750
		C_1	24/11	2.181			C_1	8/11	0.727
13	N.W. vs. S.E.	A_1	388/124	3.129*	16	S.E. vs. N.W.	A_1	240/67	3.583*
		A_2	144/42	3.427*			A_2	41/20	1.413
		B_1	6/9	0.666			B_1	59/33	1.484
		B_2	36/15	2.400			B_2	14/13	1.076
		C_1	8/6	1.333			C_1	10/4	2.500

* Significant at 5% level.

† Significant at 1% level.

whose horizontal differences in root numbers were significant in 1 to 3 of the total five horizons. Two rectangular trenches were tested similarly. Here each initial 6-foot section was divided into two sections 3 feet in length to afford a statistical basis for application of the t test. In each of these stations the two sections differed significantly only in the A_2 horizon. In these cases either the writer was fortunate in selecting walls which showed little variation or it is to be expected normally that, owing to directional effects, one wall will show less variability than that between any two walls of the same trench. It is possible that the remaining three walls of these rectangular trenches would have shown significant differences from the selected 6-foot walls.

The results from the two types of trenches have been reported together, but the square trenches were assigned more weight. Root numbers obtained from rec-

tangular trenches in bare areas were comparatively lower than those obtained from square trenches in similar areas. Numbers secured from the former trenches were probably not an accurate sample of the true root distribution. The same is true to varying degrees of other station types investigated by the two types of trenches; for example, station 7 vs. station 15, station 5 vs. station 11, etc. The results from square trenches were obviously most accurate when listed as a total of the four walls, yielding a sample 12 feet in length compared with 6 feet for the rectangular trench.

The use of square trenches affords a basis for statistical comparison of root numbers obtained in different station types within the same plant community.⁴ Observation of the basic differences of root distribution within a community on one soil type speeds the determination of the significant relationships of the same community on several soil types. The comparison may be focused on horizons, profile sections, or total profiles. Analysis of horizontal differences in root numbers between the different station types was carried out for stations 11-16.

Significant horizontal differences were obtained for stations 11, 12, and 13, with good ground cover, as opposed to stations 14 and 16, with sparse cover. Further, there were no horizontal differences in root numbers among stations 11, 12, and 13, or among stations 14 and 16, except in the C_1 horizon in the latter stations.⁵ Such local differences might be expected considering the heterogeneous distribution of the dominant tree species, with their differing types of root systems. On the other hand, station 15, with a dense society of *P. quinquefolia* as ground cover, differed in the same direction from stations 11, 12, and 13 as did bare stations 14 and 16. However, the A_2 horizon of station 15 significantly surpassed the A_2 of the two bare stations in root numbers and was equivalent in numbers to the others with abundant cover. If one assigns to the A_1 and A_2 horizons more importance than to the others, station 15 must be segregated as possessing a distinct type of root distribution. This is in accord with the distribution in the A_1 and A_2 of this station, which is conditioned by the type of root system of the dominant ground cover species and not by the apparent relative suitability of the respective horizons.

The root-volume results give further evidence of the heterogeneity of root distribution in the deciduous forest. They indicate that the upper 8 inches of the soil contain the largest volume of roots. Samples were not taken below the first foot, but it seems unlikely that greater volumes would be found at lower levels—unless exceptionally large tree roots were encountered. The amounts are approximately equal to those found by WEAVER and HARMON (19) in the prairie.

⁴ A standard error can be obtained for each trench, since each wall, by subdivision into 1-foot sections, can yield a mean observation.

⁵ In the *t* test, "difference" is intended to mean only significant differences (those at or above the 5 per cent level in FISHER'S probability table).

Earthworms, in mixing and penetrating all horizons, are obviously of benefit to the aeration, fertility, and water relations of the profiles. Such activity warrants further investigation. Cicada activity within the soil, while most vigorous during emergence of the nymphs, is favorable in providing drainage channels and in effecting better aeration, particularly of the B₂ horizon. Its effect is probably felt for a number of years in ungrazed forests.

Summary

1. The relationship of the Bellefontaine silt loam soil profile, with its inclusions, to the root development of the native species in the maple-oak forest at Wychwood, Lake Geneva, Wisconsin, was studied during the summers of 1938-1940. Stations were selected to obtain a cross-section of the varying conditions in the forest.

2. Sixteen station types were investigated by the trench method of root-distribution sampling. In six of the station types a new, square method of trenching afforded more accurate measurement of the heterogeneous root distribution than did the rectangular trenches most commonly employed.

3. Root systems of herbaceous and woody seedlings in the various station types were excavated and their relation to the upper horizons noted. Large specimens of sugar maple, red oak, and white oak also were partially excavated. Root volumes were obtained from surface cubic-foot samples in each station type. Cicada and earthworm activity was observed.

4. Greatest concentration of the smallest roots, 0.1-1.0 mm. in diameter, was in the A₁ horizon. Larger roots did not show an increase in numbers per unit horizon surface below the A horizons. Greater numbers of dead roots occurred in lower horizons.

5. On root number and percentage root-area basis, horizons A₁, A₂, B₁, B₂, and C₁ generally showed successive decrease in suitability for root development. On the same basis there were marked decreases, with two exceptions, of root numbers and area with successive 1-foot increases in depth to 3 feet.

6. Areas with sparse ground cover showed low root numbers and comparatively high percentage root areas. Stations with more abundant ground cover were characterized by larger root numbers and smaller percentage root areas.

7. Earthworm and cicada activities, particularly the former, were important factors in the soil environment. Such activities were more or less uniform throughout the woodland.

8. In all cubic-foot root-volume samples, the 0-8 inch level contained 80 per cent or more of the total root volume present in the sample. When the sample lacked roots $> \frac{1}{2}$ inch in diameter there were successive decreases in volume in passing into the 0-4, 4-8, and 8-12 inch levels.

This study was carried out under the supervision of DR. CHARLES E. OLMSTED, who suggested the problem. The writer expresses sincere appreciation to him for his interest and helpful suggestions. He is also indebted to DR. SEWALL WRIGHT for advice on the statistical treatment of root data.

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LITERATURE CITED

1. BEAMER, R. H., Studies on the biology of Kansas Cicididae. Univ. Kansas Sci. Bull. 18. No. 2. 1928.
2. BILLINGS, W. D., The structure and development of old field shortleaf pine stands and certain associated physical properties of soil. Ecol. Monog. 8:437-499. 1938.
3. COILE, T. S., Distribution of forest tree roots in North Carolina Piedmont soils. Jour. Forestry 35:121-148. 1937.
4. DARWIN, C., The formation of vegetable mould through the action of worms, with observations on their habits. John Murray, London. 1892.
5. FISHER, R. A., Statistical methods for research workers. Oliver and Boyd, London. 1936.
6. GAST, P. R., Soil profiles developed under pines and hardwoods. Jour. Forestry 35:11-16. 1937.
7. HEIBERG, S. O., and CHANDLER, R. F., A revised nomenclature of forest humus layers for the northeastern United States. Soil Sci. 52:87-99. 1941.
8. KELLOGG, C. E., Preliminary study of the profiles of the principal soil types of Wisconsin. Wis. Geol. and Nat. Hist. Survey. Bull. 77A. Soil Series 54. 1930.
9. KORSTIAN, C. F., and COILE, T. S., Plant competition in forest stands. Duke Univ. School of Forestry. Bull. 3. 1938.
10. LUTZ, J. E., ELY, J. B., and LITTLE, S., The influence of soil profile horizons on root distribution of white pine. Yale Univ. School of Forestry. Bull. 44. 1937.
11. MARBUT, C. F., Soils of the United States. In Atlas of Amer. Agr. Part 3. U.S.D.A. Bur. Chem. and Soils. Washington, D.C. 1935.
12. MARLATT, C. L., The periodical cicada. U.S.D.A. Bur. of Entomology. Bull. 71. 1907.
13. PESSIN, L. J., Effect of soil moisture on the rate of growth of longleaf and slash pine seedlings. Plant Physiol. 13:179-189. 1938.
14. SNEDECOR, G. W., Statistical methods: applied to experiments in agriculture and biology. Iowa State College Press. 1940.
15. STRANDINE, E. J., Quantitative study of the recent emergence of the periodical cicada in the Chicago area. Bull. Ecol. Soc. Amer. 20:31. 1939.
16. TURNER, L. M., A comparison of roots of southern shortleaf pine in three soils. Ecology 17:649-658. 1936.
17. U.S. DEPARTMENT OF AGRICULTURE. Soils survey of Walworth County, Wisconsin. 1924.
18. WEAVER, J. E., The ecological relations of roots. Carnegie Inst. Wash. Publ. 286. 1919.
19. WEAVER, J. E., and HARMON, G. W., Quantity of living plant material in prairie soils in relation to run-off and soil erosion. Conserv. and Surv. Div. Univ. Nebraska. Bull. 8. 1935.
20. WHITSON, A. R., and BAKER, C. E., The climate of Wisconsin and its relation to agriculture. Wisconsin Agr. Exp. Sta. Bull. 223. 1921. (Revised in 1928.)

FLORAL INITIATION IN BILOXI SOYBEAN AS INFLUENCED BY GRAFTING

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(WITH THREE FIGURES)

Introduction

There are wide differences among soybean varieties in the lengths of daily photoperiod under which they initiate flower primordia. Plants of the Biloxi variety subjected to daily photoperiods of 14 or fewer hours produce abundant flower primordia within a few weeks, but on photoperiods of 16 or more hours they do not produce sufficient flower-forming stimulus to cause floral initiation until they are many months old. The plants of several other varieties regularly initiate flower primordia when only a few weeks old, even though they are subjected to continuous light from the time they emerge from the soil (2). Some of these varieties do not continue the development of their flower primordia under continuous light, but others produce normal flowers and fruits. Batorawka and Agate are examples of this latter type.

Although these wide differences in photoperiodic response exist among certain varieties of soybeans, it seems probable that the basic mechanism causing floral initiation in the different varieties is fundamentally the same. Evidence supporting this assumption has been obtained by grafting Batorawka or Agate soybeans to Biloxi.

Literature review

Grafting has been employed as a means to study the photoperiodic response of plants by CAJLACHJAN (4), MOSKOV (11, 12), MELCHERS (9), MELCHERS and LANG (10), KUIJPER and WIERSUM (7), KUIJPER and SCHUURMAN (8), HAMNER and BONNER (6), and others. A general review of most of these papers has been made recently by CHOLODNY (5). A primary purpose in most of the studies has been to demonstrate that a stimulus causing flowering in one plant may cross a graft union and induce flowering in the other. In some experiments the two plants concerned have been of the same variety; in others they have been of different varieties, species, or even genera. Several workers have sought to show further that the flower-inducing stimulus of the one kind of plant is identical to or closely similar to that in the other. All the investigators just cited consider flowering to be a result of hormonal action.

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Only two of the papers mentioned (7, 8) deal with the grafting of soybeans. In these, plants that had been grown continuously on long photoperiods and were apparently vegetative were grafted with others that had formed flower buds as a result of treatment with short photoperiods just prior to grafting.

In the first paper (7) results are reported from twelve grafts. In six of these the scion was from the short-day plant and the stock from the long-day one; in the other six this arrangement was reversed. All the six long-day stocks produced visible flower buds or flowers, but only one long-day scion produced flower buds before the close of the experiment. No control grafts were included. The second paper (8) reports results obtained from thirty-eight successful grafts between plants grown on long and on short day, respectively, and from twenty controls in which stock and scion were both from plants grown on long photoperiod. Flower-bud formation on the long-day component of the grafted plants occurred in only five grafts, all of which were short-day scion on long-day stock.

In both these papers two varieties—Ked. 29 from Buitenzorg and a yellow-seeded variety from Vilmorin—were used. All the twelve grafts in which the long-day component flowered were of the Vilmorin variety. In eleven of these twelve, the long-day component was the stock, while in the other one it was the scion. From this the investigators concluded that the stimulus moved downward more readily than upward across the graft union. The results of this research indicate that flowering of a soybean grown on long day may be somewhat accelerated by grafting to it a part of a plant that has been subjected to short photoperiods. The data do not show, however, that flower primordia were not present on the long-day stocks when the plants were grafted.

Plants of all the yellow-seeded lots from Vilmorin have flowered earlier than varieties such as Mandell and Minsoy when tested at Arlington, Virginia. The various lots obtained from Buitenzorg, on the other hand, have been even later than Biloxi. The varieties Mandell and Minsoy have been found to initiate flower primordia under conditions of continuous light, but the flower buds develop very slowly on long photoperiod and seldom become visible (2). From this it seems probable that flower primordia were present on the Vilmorin plants used by KUIJPER, WIERSUM, and SCHURMAN, and that the earlier flowering after grafting resulted from accelerated development of such pre-existing flower primordia. Failure of flowers to form on the plants of the Ked. 29 variety following grafting would indicate that primordia were not present on the long-day components previous to grafting and were probably not formed on them as a result of it.

Material and methods

Three varieties of soybeans (*Soja max*) and four varieties of garden beans (*Phaseolus vulgaris*) were used in these experiments. The soybean varieties were

Biloxi, Agate, and Batorawka. The garden bean varieties, all of which flower profusely even on long photoperiods, were Red Kidney, Plentiful, Black Valentine, and Dwarf Horticulture. All grafts included a Biloxi component that received long photoperiods both before and after grafting. This component was used to determine whether or not a flower-inducing stimulus crossed the graft union and

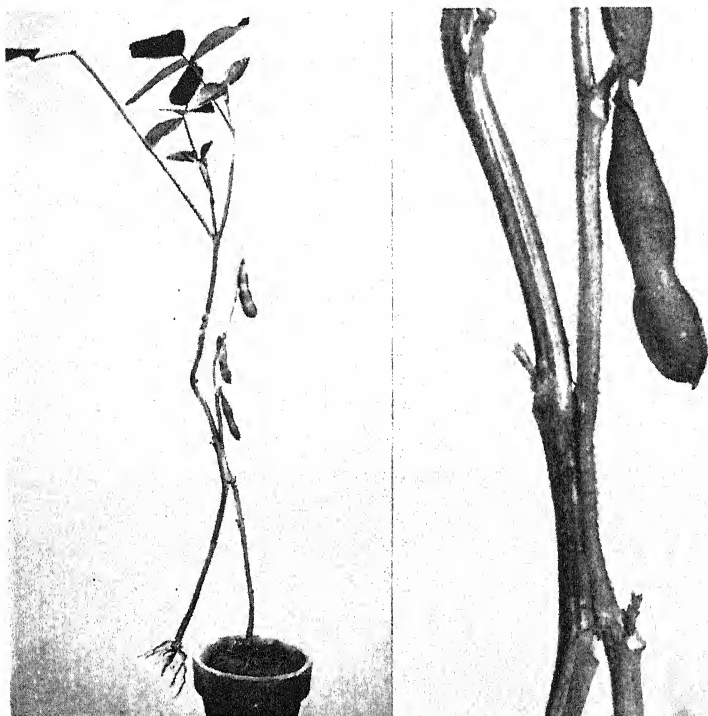


FIG. 1.—Left: approach graft of Biloxi and Agate soybeans. Right: detailed view of graft union after removal of Parafilm. Photographed about 2½ months after graft was made. Biloxi receptor initiated flower primordia soon after the grafting. A few of its flower buds finally opened but it developed no pods. Roots were removed from soil about 2 months before it was photographed.

is referred to as the receptor, following the usage of HAMNER and BONNER (6). In many of the grafts the receptor was combined with other soy or garden bean varieties, but in a few it was combined with another Biloxi plant that received short-day treatment either prior to or subsequent to grafting.

All plants were grown in 4-inch pots in the greenhouse, and on photoperiods of 17 or more hours, depending on the season. The photoperiods consisted of natural light plus Mazda light of about 50 foot-candles from sundown until midnight.

Several methods of grafting were used, including approach grafting of stems,

splice grafting of petioles and of stems, and bud grafting. The youngest plants in any experiment were 19 days old when grafted, and in no experiment were grafts made with plants older than 43 days. Approach grafts of two stems were made by removing a thin section 1-1½ inches long from adjacent sides of two stems. These cut surfaces were immediately placed against each other and wrapped with a rub-

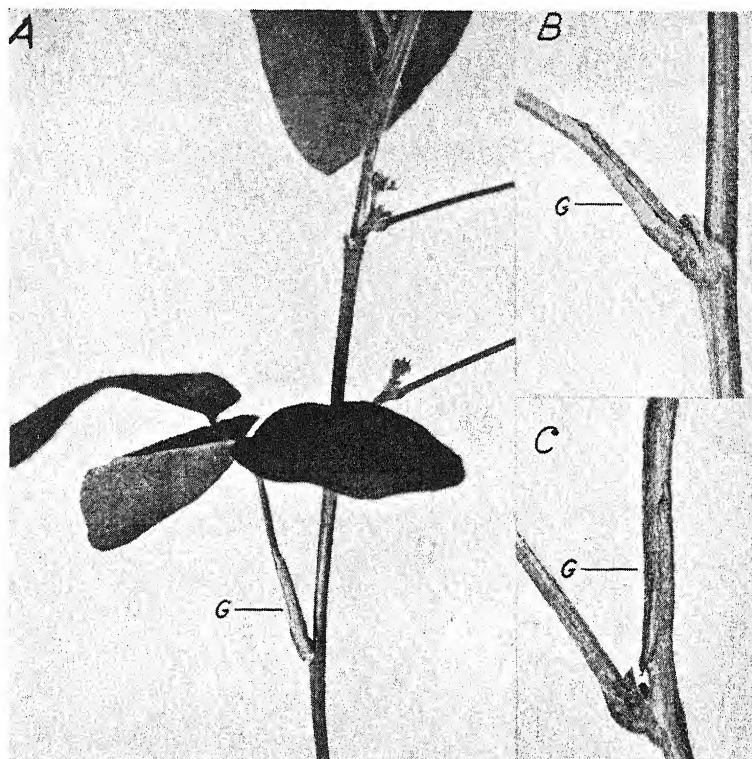


FIG. 2.—Splice grafts of petioles and of stems. *A*, Agate leaf grafted to Biloxi receptor; graft union wrapped with Parafilm. *B*, detailed view of petiole-graft union. *C*, detailed view of stem-graft union (*G*). Photographed 6 weeks after grafting.

berized paraffin tape known as Parafilm. The tape held the grafts firmly together but stretched easily as the stems increased in size (fig. 1).

In the petiole-grafting experiments, only one leaf was grafted to a single receptor plant. This leaf was always a young one and was in about the same stage of development as the leaf removed from the receptor. The petioles of the leaves were usually 1-2 inches long and the leaflets were about half their usual mature size. Both leaves were severed by slanting cuts about $\frac{3}{4}$ inch long made through the petiole, the lower end of the cut beginning on the under side of the petiole

near its base. The petioles were brought together along their cut surfaces and in most cases were bound with Parafilm (fig. 2*A*, *B*). The cuts were always made with a wet razor, and the surfaces were kept moist until the grafting operation was completed. In a few cases glass capillary tubing was used instead of the tape to hold the grafted parts in place. Immediately after the leaf was grafted, the

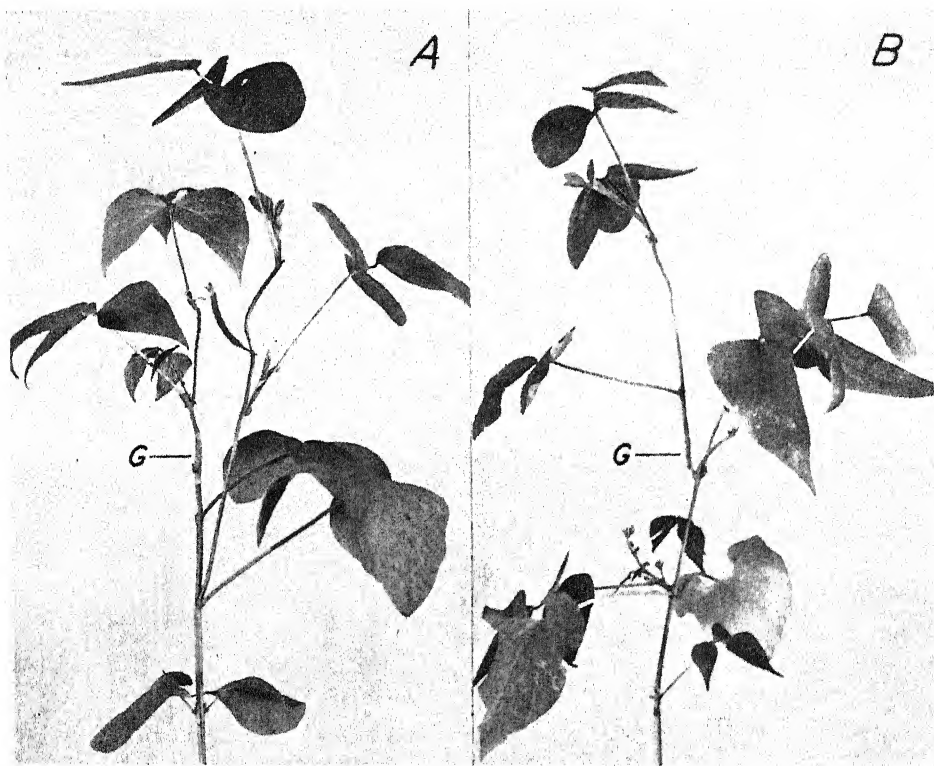


FIG. 3.—Splice grafts of Biloxi soybean and Red Kidney garden bean stems. *A*, Red Kidney scion on Biloxi stock. *B*, Biloxi scion on Red Kidney stock (*G*, graft union). Photographed 6 weeks after grafting.

plants were placed in a saturated atmosphere, where they were kept for 6 or 8 days. They were then returned to normal greenhouse conditions. At all times the plants continued to receive long photoperiods.

The plants used as stocks for the stem grafts had started to expand their second compound leaf at the time the grafts were made. Those from which the scions were taken varied in stage and type of development from one experiment to another. The scions, always $1\frac{1}{2}$ –2 inches long, included a partially expanded leaf and the terminal bud. The stems of the stocks were severed above the first com-

pound leaf. Slanting cuts were made through both stock and scion and the two surfaces brought together and securely wrapped with Parafilm (figs. 2C, 3). All stem grafts were given the same moist-chamber treatment as the leaf grafts.

A few bud grafts were made by grafting a bud of one plant on to the node of another plant where a bud had been removed. Enough extra tissue above and below the grafted bud was retained to permit wrapping without covering the bud. The top of the plant was removed in order to force the grafted bud. The plants bearing the bud grafts were placed in the moist chamber for 10 days, then removed to the greenhouse bench.

The presence or absence of flower primordia in buds of the receptors was determined in all the experiments by dissection 2-3 weeks after grafting.

Results

GRAFTS OF SEVERAL VARIETIES OF SOJA MAX TO BILOXI VARIETY

APPROACH GRAFTS.—In the spring of 1941, two preliminary experiments were conducted involving approach grafts of Agate to Biloxi soybeans. In experiment I, forty-five grafts were made; and 18 days after grafting, flower primordia were present on eleven of the receptors. In the second experiment, forty more approach grafts of the same varieties were made, and of these, nine receptors formed flower primordia in the terminal bud. The leaves were then removed from the receptors of sixteen of the thirty-one grafts that failed to form flower primordia in experiment II. All thirty-one grafts were allowed to continue development. Although the terminals of the receptors were destroyed at the time of dissection, lateral buds developed and these were dissected 2 weeks later. Flower primordia were present on twelve of the sixteen defoliated receptors at this time, while the fifteen undefoliated receptors were still vegetative.

These preliminary experiments suggested that a much higher percentage of the Biloxi plants might have formed flower primordia if the receptors had been defoliated. Experiment III was performed to test further the significance of defoliation of the receptor, and at the same time to determine whether age of the plants at the time of grafting was influential. For this experiment, 280 pairs of Agate and Biloxi plants were grafted. Twenty grafts were made on each of 14 days, beginning when the plants were 22 days old and ending when they were 43 days old. At the time of grafting, ten plants of each lot of twenty were defoliated to one leaflet.

In 120 of the 280 grafts, or in about 43 per cent of the total, flower primordia were formed on the receptor plants; the other 160 receptors remained vegetative. Age of the plants at time of grafting did not influence the results. There were minor fluctuations in the numbers of receptor plants bearing flower primordia in the groups grafted on various days, but the results showed no correlation with age.

Defoliation did not result in marked differences in number of plants producing flower primordia. Of the 120 plants that formed flower primordia, sixty-two had been defoliated at the time of grafting and fifty-eight had not been. This result is not in agreement with that obtained in experiment II, but the plants of experiment II were defoliated many days after they were grafted, whereas those of experiment III were defoliated at the time the grafts were made.

In a fourth experiment, five lots of twenty-five Biloxi-Agate approach grafts were made. One lot was not defoliated, another was defoliated at the time of grafting, and the remaining three lots were defoliated 4, 8, and 12 days, respectively, after grafting. In the undefoliated controls nine of the twenty-five receptors formed flower primordia. In the plants defoliated at the time of grafting, twelve of the twenty-five receptors formed flower primordia. In those lots defoliated 4, 8, and 12 days after grafting, seventeen, fifteen, and eighteen plants, respectively, formed flower primordia. Defoliation of the receptor, performed 4 or more days after the plants were grafted, resulted in the formation of flower primordia on more receptors than defoliation performed at the time of grafting.

The failure in all experiments of certain of the receptors to initiate flower primordia, even when they were defoliated several days after grafting, indicated that other inhibiting factors were operating. Although all the grafted plants seemed to have made good unions, there was a possibility that this was not the case. Consequently the roots of all the receptor plants were removed from the soil and allowed to dry, thereby making the survival of the receptors dependent upon the quality of the graft union (fig. 1). Thirty-five days later, only fourteen of the seventy-one flowering plants were dead, but twenty-seven of the fifty-four non-flowering ones were dead. This same trend was observed in another experiment involving forty-five grafts similarly treated. Three weeks after the roots were pulled up, fourteen of these plants were dead. These included 44 per cent of the plants that did not have flower primordia and only 24 per cent of those that had flower primordia. This correlation between high percentage of flower-bearing receptors and low percentage of fatalities among them indicates that the quality of the graft union has some influence on the transmission of a flower-forming stimulus from Agate to Biloxi.

It seems evident from the preceding experiments that in a considerable percentage of cases a flower-forming stimulus from an Agate soybean may cross an approach graft union and result in floral initiation in a Biloxi stock. It would therefore appear possible that, if two Biloxi plants were grafted together, a flower-forming stimulus induced in one by placing it on short photoperiods would move across the graft union and cause floral initiation in the other. Several experiments of this type, involving a total of eighty approach grafts in which both donor and

receptor were of the Biloxi variety, were performed. Forty of the donors were given induction treatment with short photoperiods prior to grafting and forty of them after the grafts were made. Of the donors that were induced prior to grafting, thirty were given four short photoperiods and ten were given seven short photoperiods. Of those induced after grafting, ten were given short photoperiods for 8 days and thirty for 14 days. The receptors in these experiments were treated in three ways: some were not defoliated, others were defoliated to one leaflet at the time of grafting, and still others were defoliated several days after grafting. In all these grafts, only one receptor initiated flower primordia. This plant had been undefoliated and its donor had received eight short photoperiods after grafting.

PETIOLE SPLICE GRAFTS, STEM SPLICE GRAFTS, AND BUD GRAFTS.—In two experiments performed at different times during the fall of 1941, fifty-five Agate leaves were grafted to separate Biloxi receptors (fig. 2). Fifty-three of these grafted leaves survived, enlarged to about their customary size, and remained on the plants as long as ungrafted leaves of similar age. Flower buds were initiated on all fifty-three receptors but were not formed on the other two plants on which the Agate leaf failed to survive. As controls for these experiments, noninduced Biloxi leaves were grafted to Biloxi receptors. At the same time Biloxi leaves that had been induced 7 and 12 days were grafted to Biloxi receptors of two other lots, respectively. Floral initiation occurred on none of the receptors to which either induced or noninduced Biloxi leaves were grafted.

At the time of dissection, 2 weeks after grafting, there were many more flower buds on all the Biloxi receptors to which a single Agate leaf had been splice grafted than were present on the receptors to which an entire Agate plant had been approach grafted. The flower primordia on receptors of approach-grafted plants usually occurred at only a few nodes, and the younger buds that differentiated above these were vegetative in every case. On the receptors to which Agate leaves were splice grafted, all the buds differentiated after the first flower primordia were formed became flower buds.

Since abundant flower buds were present on Biloxi receptors 2 weeks after Agate leaves were grafted to them, the stimulus causing their initiation must have reached the meristems of the receptors very quickly. An indication of the length of time necessary for an Agate leaf to remain grafted to a receptor plant in order to result in floral initiation was obtained by removing the Agate leaf from the receptor at various times after grafting. For the first experiment of this type an Agate leaf was grafted to each of the thirty Biloxi plants. After 1, 2, 3, 4, 6, and 8 days, the grafted leaf was removed from lots of five receptors. At the time of dissection, flower primordia were present on three, four, and five of the

receptor plants on which an Agate leaf had remained for 4, 6, and 8 days, respectively, but were absent on receptors from which the Agate leaf had been removed after 1, 2, or 3 days.

In another experiment, also with thirty grafted Biloxi plants, the Agate leaves were removed from ten of the receptors after 3, 4, and 6 days, respectively. No flower primordia were formed on the ten receptors of the 3-day lot, but they were present on four and six receptors of the 4- and 6-day lots, respectively.

Batorawka soybean leaves are as effective as Agate leaves in producing floral initiation on Biloxi receptors. A Batorawka leaf was grafted to each of thirty receptors, and on all the receptors flower primordia were initiated.

Flower-bud initiation occurs on Biloxi receptors following splice grafting of stems as readily as following splice grafting of leaves. Twenty-eight splice grafts of stems of Biloxi and Agate were made. In eighteen of these the Biloxi receptors were the scions and in ten they were the stocks. Flower primordia were formed on the receptor components of all twenty-eight grafts. Likewise, in twenty Biloxi-Batorawka stem splice grafts, flower primordia were formed on all Biloxi receptors. In ten of the grafts the receptor was the scion and in the others it was the stock. No flower primordia were formed in the terminal buds of ten Biloxi-Biloxi stem grafts in which both stock and scion were grown continuously on long photoperiod.

A few Biloxi buds were grafted to Agate plants. About half the grafted buds survived. These developed into short branches, in the buds of which flower primordia were formed.

GRAFTS OF SEVERAL VARIETIES OF *PHASEOLUS VULGARIS* TO BILOXI SOYBEAN

A flower-forming stimulus passes readily across certain types of graft unions between Agate or Batorawka soybean and Biloxi and results in the initiation of flower primordia on the Biloxi receptors. Grafting experiments were conducted to determine whether or not a similar stimulus originating in plants of *Phaseolus vulgaris* might cross a graft union to a Biloxi plant and cause flowering. The varieties of garden beans used were Plentiful, Black Valentine, Red Kidney, and Dwarf Horticulture. Flowering in these varieties is influenced very little by length of photoperiod.

The earliest stages of flower primordia are initiated on many varieties of garden beans soon after the seedlings emerge from the soil. Grafts of Biloxi soybeans with garden beans are almost invariably successful, and the scion—whether Biloxi soybean on Red Kidney bean or Red Kidney on Biloxi soybean—makes vigorous growth (fig. 3). Likewise, petiole splice grafts of garden bean leaves to Biloxi soybean plants nearly always succeed. No flower primordia, however, were formed

on any of the sixty Biloxi receptors to which *Phaseolus* leaves were grafted, or on any of the thirty Biloxi receptors to which *Phaseolus* stems were grafted.

Since the scions (in the case of the stem splice grafts) and the leaves (in the case of the petiole splice grafts) become firmly attached, maintain their turgidity, and make further growth after the grafts are made, it seems evident that the translocation of water and probably of mineral nutrients occurs across the graft union. There is evidence, however, that elaborated food materials move across the graft union with difficulty or not at all. For example, a Red Kidney bean leaf that has been grafted to a Biloxi soybean for a week or more does not lose its accumulated starch when left in the dark for many days. Control Red Kidney leaves growing on Red Kidney plants lost most of their starch content the first night. Moreover, an abscission layer eventually develops across the base of the Biloxi petiole to which such leaves are grafted. The stump of the Biloxi petiole turns yellow while the petiole of the *Phaseolus* leaf remains green. The loss of color and abscission of the Biloxi stump occur at about the same time as in similar stumps of petioles to which no *Phaseolus* leaf is grafted. This is in contrast to the behavior of Agate or Batorawka leaves splice grafted to Biloxi. Such leaves remain attached to the Biloxi plant many weeks longer than *Phaseolus* leaves similarly grafted.

Root formation on *Phaseolus* scions constitutes further evidence of lack of food transfer across Biloxi-*Phaseolus* graft unions. These roots form at the base of stem scions and at the lower end of the *Phaseolus* petiole in the petiole splice grafts.

Finally, continued development of *Phaseolus* scions on Biloxi stocks or of Biloxi scions on *Phaseolus* stocks depends upon the presence of leaves on the scion. If all of the leaves are removed from the scion, whether it is Biloxi or *Phaseolus*, the scion fails to develop new leaves. But if a small portion of only one leaflet is left on the scion, the scion continues to grow and develop new leaves.

Discussion

The flower-inducing stimulus of a single Agate leaf grafted to an entire Biloxi plant was sufficient to cause flower-bud initiation in all cases. The flower-inducing stimulus in the two varieties must therefore be the same. In Biloxi the formation of the stimulus does not occur unless the photoperiod is less than 14 hours, while in Agate it is formed even under continuous light.

When Biloxi and Agate plants were approach grafted, however, flower-bud initiation resulted in less than 50 per cent of the Biloxi plants in most experiments. Every Agate plant used for the approach grafts had several leaves, each of which was capable of supplying a flower-inducing stimulus. A more than adequate stimulus, therefore, must have been produced by the Agate plants. Failure of the re-

ceptors to form flower primordia evidently resulted from inadequate transfer of this stimulus to them. Defoliation of the receptors several days after grafting increased the percentage of those initiating flower primordia. Evidently defoliation aided the transfer of the stimulus from the Agate to the Biloxi plant. Such treatment decreased the amount of organic substances synthesized in the defoliated plants and presumably changed the translocation gradient in such a way that an increase in net movement of materials from the Agate to the Biloxi component may have resulted.

Similar results from defoliation have been obtained in other photoperiodic experiments (1), in which two-branched Biloxi soybean plants were used. In these experiments one branch received several short photoperiods while the other was subjected continuously to long photoperiods. Floral initiation occurred abundantly on the short-day branches but occurred only on those long-day branches from which the leaves had been removed at the time the induction treatments were given. The results of this experiment could likewise be explained on the basis of a modified translocation gradient. On the other hand, it could be assumed that the effects of the flower-inducing stimulus of the donor leaves may have been nullified by some inhibiting substance produced by the leaves of the receptors which were on long photoperiods, or that the stimulus from the donor leaves was absorbed by the leaves of the receptors to such an extent that insufficient amounts reached the growing point with sufficient intensity to cause floral initiation—in spite of the fact that all but one of the Biloxi leaves were still present on the plant.

Biloxi leaves that had been subjected to several short days before they were grafted failed to induce the formation of flower primordia on receptor plants. If flower primordia had been formed on the receptors this would have meant that the grafted leaf had undergone some change resulting from the induction treatment that enabled it to continue liberating a flower-inducing stimulus after it had been returned to long photoperiods. Failure of the grafted leaves to induce flower primordia on the receptors is in agreement with the results previously obtained in which it has been shown that the flower-inducing stimulus does not continue after the plants are returned to long photoperiods.

Although all donors produced abundant flower buds in the Biloxi-Biloxi approach grafts, only one receptor formed flower primordia. The flower-inducing stimulus was evidently present in the donors but for some reason failed to reach the terminal meristems of the receptors in sufficient amount to result in flower-bud formation. The reasons for this are not known.

It has been shown previously (3) that the stimulus resulting in floral initiation in the Biloxi soybean will readily move either up or down the plant. Further evidence that this is true was obtained from the stem splice-grafting experiments between Agate and Biloxi. Since flower buds formed on the receptors, whether they

were scions or stocks, it is evident that the flower-inducing stimulus crossed the graft union in either direction. KUIJPER and SCHUURMAN (8) did not observe an upward translocation of the stimulus in the soybean varieties they grafted.

Summary

1. Biloxi plants or parts of Biloxi plants, subjected continuously to daily photoperiods of 17 or more hours, were used as one component of all grafts reported in this work. Plants of this variety do not develop sufficient flower-forming stimulus on long photoperiods to cause floral initiation. They therefore served to determine whether or not a flower-inducing stimulus was transmitted across the graft unions. They are referred to as receptors and the plant or plant parts grafted to them as the donors.

2. The donor components were from Agate, Batorawka, or Biloxi varieties of *Soja max*, or from Red Kidney, Plentiful, Black Valentine, or Dwarf Horticulture varieties of *Phaseolus vulgaris*.

3. Methods of grafting employed were approach grafting of stems, splice grafting of petioles, splice grafting of stems, and bud grafting.

4. A total of 490 Agate-Biloxi approach grafts were made and all formed strong unions. Approximately 50 per cent of the Biloxi receptors formed flower primordia. This percentage was somewhat greater if the receptors were defoliated a few days after they were grafted.

5. Eighty Biloxi-Biloxi approach grafts were made. Flower primordia were formed on no receptors whose donors received long photoperiods continuously, and on only one receptor whose donor received short photoperiods after it was grafted.

6. Eighty-five Agate or Batorawka leaves were grafted to Biloxi receptors. Flower primordia developed on all eighty-three receptors on which the grafted leaf survived.

7. Formation of flower primordia on Biloxi receptor plants to which an Agate leaf was grafted occurred only when the grafted leaf remained on the receptor 4 or more days.

8. Flower primordia developed on all the Biloxi receptors of the forty-eight Agate or Batorawka stem grafts made. The flower-inducing stimulus passed either up or down through the graft union, depending upon whether the receptor was the scion or the stock.

9. Thirty grafts of Biloxi leaves on Biloxi receptors, and twenty of Biloxi stems to Biloxi receptors, were made; all lived, but flower primordia were formed on no Biloxi receptors. In some of these grafts the Biloxi donors had received long photoperiods at all times; in others they had received various numbers of short photoperiods prior to grafting.

10. Sixty leaf grafts and thirty stem grafts of *Phaseolus* bean to Biloxi soybean receptors were made. Of these, fifty-nine of the former and twenty-seven of the latter survived, but flower primordia were formed on no Biloxi receptors.

11. Water and probably mineral nutrients move across *Phaseolus*-Biloxi graft unions readily, but elaborated foods do not appear to cross such unions.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic perception in Biloxi soybeans. *BOT. GAZ.* 100:374-387. 1938.
2. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic responses of several varieties of soybeans. *BOT. GAZ.* 101:341-365. 1939.
3. BORTHWICK, H. A., and PARKER, M. W., Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. *BOT. GAZ.* 101:806-817. 1940.
4. CAJLACHJAN, M. CH., Motion of blossom hormone in girdled and grafted plants. *Compt. Rend. (Doklady) Acad. Sci. U.R.S.S.* 18:607-612. 1938.
5. CHOLODNY, N. G., The internal factors of flowering. *Herbage Reviews* 7:223-247. 1939.
6. HAMNER, K. C., and BONNER, JAMES, Photoperiodism in relation to hormones as factors in floral initiation and development. *BOT. GAZ.* 100:388-431. 1938.
7. KUIJPER, J., and WIERSUM, L. K., Occurrence and transport of a substance causing flowering in the Soya bean (*Glycine Max* L.). *Proc. Roy. Acad. Sci., Section of Sciences. Amsterdam* 39:1114-1122. 1936.
8. KUIJPER, J., and SCHUURMAN, J. J., Proeven over het transport van een bloeiveroorzakende stof bij soja en aardappelen. *Landbouwkundig Tijdschrift* 50:583-589. 1938.
9. MELCHERS, G., Die Wirkung von Genen, tiefen Temperaturen und blühenden Pfropfparten auf die Blühreife von *Hyoscyamus niger* L. *Biol. Zentbl.* 57:568-614. 1937.
10. MELCHERS, G., and LANG, A., Weitere Untersuchungen zur Frage der Blühormone. *Biol. Zentbl.* 61:16-39. 1941.
11. MOSKOV, B. S., Blooming of short day plants in a condition of continuous illumination as a result of grafting. *Bull. Appl. Bot. Gen. and Pl. Breed. Ser. A, Supplement.* 21:145-156. 1937.
12. MOSKOV, B. S., Photoperiodism and a hypothesis as to hormones of flowering. *Compt. Rend. (Doklady) Acad. Sci. U.R.S.S.* 15:211-214. 1937.

GROWTH AND DEVELOPMENT IN RANGE GRASSES. II. EARLY DEVELOPMENT OF *BOUTELLOUA CURTIPENDULA* AS AFFECTED BY DROUGHT PERIODS

CHARLES E. OLMSTED

Introduction

Studies on the development of *Bouteloua curtipendula* as affected by watering frequency have been reported previously (9). In conjunction with that research it seemed desirable to determine the effects of severe soil drought upon seedlings watered at various frequencies, at different developmental stages. Both soil and atmospheric drought periods may be considered part of the normal environment during seedling establishment of most range grasses. In cereals, the occurrence of critical periods in development, during which limited water supply has greatest effect, has long been known (1, 7). In certain spring wheats, AAMODT and JOHNSTON have shown that superior drought resistance is associated with (a) ability to evade early periods of drought; (b) rapid development of primary root systems when young, both in branching and number of primary roots; and (c) superior capacity to endure drought without permanent injury. In perennials, equally important must be the ability of certain parts, usually underground or nearly so, to remain dormant but alive during periods of soil drought which cause death or dying back of tops (2).

Few workers on range grasses, even when recording death of parts (11), have considered carefully the morphological pattern of individual species in relation to the problems of drought resistance or the possible effects of drought upon this pattern. In general, only those anatomical modifications considered to be xeromorphic, or the rate and depth of root penetration and root branching, have been considered. The differential availability of a limited water supply to different organs within a plant, in conjunction with differential protection from—and resistance to—desiccation, may lead to interesting developmental patterns. In maize (6) it has been suggested that stunting effects tend to be most serious during the formative period; that is, during the first 30–40 days of the plant's life, when the developmental pattern is laid down. In certain fruit trees, drought conditions during the period of floral differentiation may result in excessive flower-bud formation (4), while in cotton the flower buds are shed (5). GLENDENING (3) reported flowering by *Heteropogon contortus* in 7–36 days following resumption of watering after the subjection of 7-weeks-old plants to a drought period of 6 weeks. Plants, grown at medium levels of water supply preceding this period, flowered earlier

than those first subjected to dry or wet conditions. He gave no data for plants not subjected to drought.

In addition to gross observations on drought effects, including death of parts, some attempt was made in the present study to follow the rate and degree of development of individual organs initiated just before, or early in, a drought period, together with any correlative effects produced in the plant as a whole when watering was resumed. As previously indicated, *Bouteloua curtipendula* was chosen for study because of its considerable increase in importance in various parts of the central grassland region during the early years of the drought period. A study of its early development in relation to water supply and drought might aid in interpreting this increase.

Methods and environmental conditions

Procedure and general growth conditions during the experiment, except soil moisture, have been reported (9). Plants were grown from seed in 2-gallon glazed crocks, and were thinned to ten plants per crock. Water was supplied to three basic series in an amount equivalent to 0.4 surface-inch at intervals of 3, 6, and 12 days. From these series, four or five crocks of each were set aside to dry when plants were 18, 30, and 42 days old. In a fourth series, watered every 20 days, five pots each were placed on drought after 26 and 46 days. In all cases, the beginning of drought periods coincided with the end of a normal watering interval, with the measurement of soil moisture, and with root and top development in the four basic series (9, tables 1, 3-6), the latter series having been set up on November 27, when the plants were 6 days old. All series were given the regular watering of 0.4 surface-inch on the first day of their drought periods. This produced the wettest condition at the beginning of the dry period in the series previously watered most frequently (at intervals of 3 days), while driest conditions were found in those first watered at 20-day intervals. In the latter, both at 26 and 46 days, soil-water content had been reduced almost to the permanent wilting percentage. Among pots with a 3- or 6-day watering interval preceding drought, those placed on drought at 42 days were also much wetter than those allowed to dry earlier. These conditions were not disadvantageous, because the larger plants in both the older and wetter series reduced the water content during drought much more rapidly than did the smaller plants which began drought under seemingly much drier conditions. The net result was probably to impose more nearly the same intensity of drought on all series than could have been done in any other way under the experimental conditions. After 48 days of drought treatment, soil-moisture content in all series and ages was reduced below the permanent wilting percentage, and ranged from 0.1 to 0.5 per cent above the hygroscopic coefficient of 0.8 per cent. As judged by soil-water content, duration and degree of wilting,

death of foliage, and length of time required for recovery, water deficit became somewhat more acute in all series placed on drought when 42 days old than in the others, and was least severe in those where drying started at 18 days.

After 48 days of drought, plants from one crock in each series were washed out for measurement. One pot of each was left dry to complete total drought periods of 88, 80, 76, 69, and 65 days for series allowed to dry when 18, 26, 30, 42, and 46 days old, respectively. These latter pots were then watered amply to test survival and amount of recovery during the following week. The remaining three pots in each series were given 0.4 inch of water after 48 days of drought, and one pot of each was washed out after 3 days for a record of renewed growth. The remaining two pots were watered (0.4 inch) at intervals of 3 or 12 days until the close of the experiment, to allow recovery under both wet and dry conditions. All remaining plants were harvested at 119 days. Hereafter in referring to treatments, numerals such as 3-18-48-3 will indicate, in order, plants watered at intervals of 3 days until 18 days old, followed by a drought treatment of 48 days, after which watering was resumed at 3-day intervals until 119 days old. Total amounts of water supplied to series left on drought for 48 days ranged from 9.6 surface-inches in 3-18-48-3, 3-30-48-3, and 3-42-48-3 to 2.8 in 20-26-48-12 and 20-46-48-12. Several other treatments received the same total amounts of water, including 12-18-48-12, 12-30-48-12, and 12-42-48-12 (3.2 inches). Harvesting and measurements of growth and development followed the procedure outlined in the previous report (9)

Results

PRIMARY ROOT SYSTEM

The imposition of a drought period, if not too severe, probably tended to prolong the life of the primary root system. It was still alive in the great majority of the 220 plants, examined at 119 days, in the various treatments involving 48 days of drought. This is in contrast to its relatively early death in the continuously wet series, previously reported (9). It was found dead most frequently in those series, such as the 3-42's or 6-42's, in which frequent watering and greater age had allowed establishment of a number of adventitious roots from the primary axis before the drought period. It was the only functional absorbing organ in thirty-three plants, all alive, of the 220, and in fourteen others was supplemented only by one to four roots developed from secondary axes after the resumption of watering at 3-day intervals. Of twelve plants, however, which either had died by the end of the 48-day drought or had failed to recover on a 12-day watering interval, nine had not established adventitious roots, suggesting the inadequacy of the primary root system alone during severe stress in some plants. In three of the nine, death actually resulted from breaking of the mesocotyl. The forty-two plants, either living or dead, which had failed to establish adventitious roots were found in

fourteen of the twenty-two treatments, but only two of these had been watered at 3-day intervals preceding drought.

In those pots subjected to drought for periods longer than 48 days, only fifteen plants out of 107 were alive when harvested. They were found in four pots—3-18-88, 3-30-76, 6-30-76, and 3-42-69. Of the thirty-eight plants in these four pots, fourteen of the fifteen alive had established one or more adventitious roots. Of the thirty-nine plants placed on drought when 18 or 26 days old, five of the six still alive at 119 days showed establishment of the first adventitious roots to be formed, while only the primary root had become functional in the thirty-three which had died. Of the thirty placed on drought at 30 days, the eight remaining alive had a range of one to five established adventitious roots in contrast to none to three in the dead plants. These data illustrate the importance of early establishment of adventitious roots in seedling survival of severe drought.

The influence of drought periods on degree of branching and penetration of the primary root system was impossible to determine, since these roots are so slender as to undergo considerable breakage in removal from soil. It was observed generally, on plants washed out immediately at the close of drought periods, that differentiation of root tips in soil had apparently occurred to within a fraction of a millimeter from the tip, in both primary and secondary root systems, as judged by root-hair production and other criteria. These tips renewed growth very slowly from the residual meristem and in most cases had elongated only 2 mm. or less on plants washed out 3 days after watering was resumed. In some cases no new growth was apparent, even on tips which still seemed alive.

PRIMARY STEM, LEAVES, AND TILLERS

Death of primary leaves and of those on secondary axes proceeded from the tips downward, from the outer to the inner margin of the convolutely-rolled wilted leaves, and from lower to higher positions on each axis, until death of stem tissues occurred. There were no consistent differences in percentage of dead foliage among the different treatments. In practically all plants subjected to drought for 48 days, almost all foliage had died which was not protected by a favorable position in vernalion upon wilting and rolling. Generally only the one or two leaf primordia which had not yet partially emerged from the tube of leaf bases, and the basal portions of sheaths or inner halves of the convolutely-rolled blades of the next two or three older leaves, remained alive on the various axes. Protection afforded the growing points seemed fairly effective, because in only a few of the plants alive after the 48-day drought had any primary or secondary stem tips died. In these few plants, in those remaining alive after longer drought periods, and in those which failed to recover, death occurred first in stem meristems in the most exposed positions above the ground. This condition was often associated with the earlier

death of segments of elongated internodes no longer protected by leaf sheaths. In cereals, the period of "shooting" is regarded as one in which water deficit is most critical (1, 7), often leading to death of the primary axis, with consequent delay in heading and reduction of yield after recovery by development of lateral axes.

After the resumption of watering, survival became apparent within 24 hours in most plants by the unrolling of the living portions of leaves and the renewal of growth by leaf and stem meristems, resulting in the rapid emergence of fresh green tissue through the mass of surrounding dead leaves.

The pattern of top growth was not changed markedly by drought, in correlation either with age at time of drought or with previous watering interval, except in those few plants in which stem meristems had died. There was some suggestion of the maturation of one or two more basal unelongated internodes, on both primary and secondary axes, in those plants in which shooting would normally have occurred during the drought period than in the control series previously described (9). In other cases, as in 3-30-48-3, there was marked reduction in length of the second or third elongating internode, which differentiated and elongated during the drought period, compared with its length in plants not subjected to drought. Such stunting apparently was not deleterious in further growth of the same axis.

In some individual plants in which rapid differentiation and growth of the first or second primary tillers should have occurred during the drought period, a suggestion of stunting was noted. A larger proportion of first primary tillers had remained small, and there was more of a tendency for the third tiller to be largest than in the series continuously watered. Based on averages, however, the second tiller was taller than the first and third in all treatments at 119 days.

In analyzing the numerous criteria of growth and differentiation of the tops, the twenty-two treatments with a drought period of 48 days were arranged in sequence of total water supplied, together with the four series not subjected to drought. The relative rank of the different treatments, based on living plant averages for numerous growth measurements in the 119-day plants, was likewise determined. Any striking deviations in the latter from the sequence based on total water supplied were easily seen. In general, it was found that drought at different ages, and in conjunction with previous and subsequent divergence in watering frequency, produced no outstanding deviations in rank based on different growth data from that based on total water supplied. Criteria of size, such as height of plant or tillers, or length and number of elongated internodes, usually showed a wider range of values than did those of organ differentiation, such as numbers of primary leaves, tillers, etc. This suggests a greater relative effect of drought on enlargement than on organ differentiation, if meristems remained alive.

Average height at 119 days, in plants subjected to 48 days of drought, ranged from 50 cm. in 6-18-48-3 (8.8 inches of water) to 22 cm. in 20-26-48-12 (2.8

inches). Plants with the same total water supply were approximately equal in height; for example, 3-18-48-3, 3-30-48-3, and 3-42-48-3 (9.6 inches of water) averaged 45, 46, and 44 cm. tall, respectively. Plants 12-18-48-12, 12-30-48-12, and 12-42-48-12 (3.2 inches) averaged 30, 29, and 29 cm. Numbers of primary leaves in the same series were 10.0, 10.2, 9.9, and 8.2, 8.1, and 7.2, respectively. Leaf number ranged from 10.6 to 7.2 in 12-18-48-3 and 12-42-48-12. Average number of elongated internodes on the primary axis ranged from 3.8 in 6-18-48-3 to 0.2 in 20-46-48-12, while in the two groups just listed the averages were 3.7, 3.3, 3.3, and 1.7, 1.3, 0.6.

Average length of the second primary tiller ranged from 36 cm. in 3-18-48-3 to 10 cm. in 20-26-48-12, while leaf numbers were 9.9 in 12-18-48-3 and 6.8 in 20-46-48-12. Heights in the two groups with equal water supply were 36, 30, 27, and 13, 14, 13 cm., while leaf numbers were 9.2, 9.2, 8.9, and 7.6, 7.7, 7.0.

Highest values for (a) number of primary tillers over 15 cm. long, (b) number of primary tillers showing internodal elongation, (c) height and (d) number of leaves of largest primary tillers, (e) number of secondary tillers and (f) those over 15 cm. long, and (g) tertiary tillers were 5.2, 2.2, 41 cm., 9.9, 25.7, 5.3, and 21.4. Lowest values for the same criteria were 2.7, 0, 12 cm., 6.6, 12.6, 0, and 0, respectively.

In general, values in the different treatments for all growth measurements fell into or near the sequence based on total water supply. Treatments with most consistent departure from this sequence were 3-42-48-3 and 3-42-48-12, showing lower rank in most growth criteria, and 6-18-48-3, 12-30-48-3, 6-42-48-3, 12-42-48-3, and 20-26-48-12, with higher rank in growth than in sequence of water furnished. In the first two, the lower values may be attributed to inability to use efficiently the abundant water supplied before drought, to a somewhat greater retardation in growth during the drought period owing to the relatively early exhaustion of the water reserve by these largest plants, and possibly to an actual lowered physiological drought resistance owing to the early abundant supply of water. In the other five treatments, some one or all of these three factors may have operated in the opposite direction to allow the more effective development.

SECONDARY ROOT SYSTEM

The plan of initiation of adventitious roots on the primary axis has been described in detail (9). Numbers initiated near lower nodes during the previously reported study tended to be correlated with frequency of watering. Near the upper nodes, and on secondary axes, any such tendency was counteracted in part by a tendency toward stimulation of primordium initiation when older primordia failed in establishment. In the drought series, both of these tendencies naturally became effective, the drought conditions tending to inhibit initiation as well as establishment, while the failure of the latter during drought apparently stimu-

lated production of new primordia when water again became available. Rate of new production in individual plants often seemed to be inversely proportional to the number previously established, although regulated also by the adequacy of water supply following drought, which controlled further shoot development.

In the great majority of all treatments with 48 days of drought, major deviations in rank at 119 days—from that based on water supply—were found in practically all measurements of root development. The data included averages for: total numbers of primordia initiated on stems; percentage remaining alive; numbers produced on primary and secondary axes and near each node of the primary axis; total numbers of established roots (living roots over 50 mm. long) and totals on main axis and primary tillers; and numbers and percentage of established roots near each node of the primary axis.

Total numbers of root primordia produced showed strong correlation with frequency of watering following drought, those plants with the more frequent watering mostly outranking those watered on 12-day intervals. Values in the former group ranged from 42.7 in 6-18-48-3 to 28.7 in 6-42-48-3; and in the latter, with two exceptions, from 28.3 in 12-18-48-12 to 15.2 in 12-42-48-12. The exceptions, 6-18-48-12 and 20-26-48-12, produced 34.4 and 34.6 primordia, respectively. The latter pot was the only one in which all the ten plants failed to establish any adventitious roots, leading to the most extreme production of primordia in relation to the limited amount of water supplied. In the other, only three of ten plants established one adventitious root each before being placed on drought. In the two larger groups, rank in total primordium production was displaced upward, from that accorded by water supply, in all treatments in which imposition of drought at 18 days, or infrequent watering prior to drought in older plants, had prevented adventitious root establishment. Conversely, rank in primordium production was shifted downward in all treatments favoring early establishment of adventitious roots.

The percentage of primordia and roots, alive at 119 days, ranged from 74 in 6-42-48-3 to 36 in 6-18-48-12. It was obviously influenced both by death of roots from drought and other causes and by the relatively high numbers of living primordia most recently initiated in certain treatments. These two values often compensated each other in the percentage figure.

Total numbers of adventitious roots initiated on the main axis ranged from 14.6 in 12-18-48-3 to 10.1 in 6-42-48-3 and 8.2 in 12-42-48-12. This value was related to a complex of factors: the degree of maturity of all crown nodes and internodes (in some plants, grown under driest conditions, primordia were not yet differentiated near the higher nodes); the tendency of both a drought period and infrequent watering to increase the number of internodes which do not elongate with consequent increase in number of nodes near which primordia are normally

differentiated; and the tendencies for larger numbers to be initiated by plants with a favorable water supply, counteracted by the stimulating effect—both in time and numbers initiated near higher nodes—of previous failure in establishment. Actual numbers of roots initiated on the primary axis could be interpreted only by reference to the differential importance of these factors in the various treatments.

Numbers of root primordia initiated on secondary axes showed approximately the same relation in the different treatments to the sequence based on water supply as did the total numbers per plant. They ranged from 25.4 in 6-18-48-3 to 6.4 in 6-30-48-12.

Live established roots over 50 mm. long on the main axis ranged from 5.2 in 6-42-48-3 to 0 in 20-26-48-12. With the exception of the latter, and 12-42-48-12 (3.6 roots) and 3-42-48-12 (2.9 roots), such roots in all other treatments—with a 12-day watering frequency following drought—ranged from 2.2 to 0.6. In very few of these plants were any adventitious roots established on either primary or secondary axes following drought, emphasizing the need in this species, previously reported, of at least 3 consecutive days of soil-surface wetness for such establishment. Plants watered at 3-day intervals following drought, except 20-46-48-3 (1.8 roots) and 20-26-48-3 (1.1 roots), possessed from 5.2 to 2.6 established roots at 119 days. In this group of treatments one or more roots per plant were established following the resumption of watering. In both groups, the average number of adventitious roots established before drought increased consistently with age at drought in all frequencies of watering, and in general, at each age, with increasing frequency of watering.

Established live roots on tillers were found, with rare exceptions, only on plants watered at 3-day intervals following drought, ranging in number from 5.4 in 3-18-48-3 to 1.1 in 3-42-48-3 and 20-46-48-3. Those earliest subjected to drought consistently had the highest number of established tiller roots in contrast to those allowed to dry when older. This fact is related to the coincidence of the resumption of watering in the former with (or near) the time when first-formed tillers had reached a developmental stage, allowing vigorous root initiation and some successful establishment. In plants placed on drought when older, many primordia, initiated just before or during the early stages of drought, had died before watering was resumed or were too weak to make effective growth in the somewhat arid aerial environment characteristic of the latter phases of the experiment. In practically all plants watered at 12-day intervals following drought, tiller roots failed completely, although initiated in considerable numbers. Death occurred usually because the rate of soil drying exceeded their rates of penetration.

The length of time during which initiated primordia, still buried in stem tissues, retained their capacity for development was shown by analyzing the data with

respect to time of production and establishment of roots near the successive nodes on the primary axis. For example, plants placed on drought at 18 and 26 days had commonly initiated primordia near the coleoptilar (second) node, and in some cases near the third node as well. The former primordia, especially, grew in response to the application of water at this time; and—in those plants previously watered at 3- or 6-day intervals—often became established. Primordia near the third node failed to grow at this time in most treatments, or, having done so, failed in establishment during the beginning of the drought period. During the gradual increase in internal water deficit, primordia were produced successively near the higher nodes, so that at the conclusion of the drought period (plants 66 and 74 days old) roots had been initiated near all nodes up to and including the fifth, and in some cases, the sixth. Upon resumption of watering, however, it was found that only those primordia near the higher nodes, particularly the fifth, were capable of effective growth and successful establishment. The primordia near the third and fourth nodes had often died, still buried in apparently living stem tissue; or, if still alive, seemingly showed cell maturation to the tip and incapacity for growth. In other cases it was obvious that great amounts of mechanical tissue had differentiated from the surrounding stem cells, possibly restricting the activity of the roots.

On plants placed on drought when older, primordia near somewhat higher nodes failed to develop into functional roots, for the reasons already suggested. Thus, plants placed on a 48-day drought at 42 days, after frequent watering, followed by watering at 3-day intervals, usually showed functional established roots near the second and third, and occasionally the fourth, nodes. Most primordia near the fourth and fifth nodes died, either with or without elongation, during the drought period. New roots grew, after watering, from near the sixth, occasionally the fifth, nodes; but so slowly in many cases as to dry before they could become established. In addition to moist soil, a humid atmosphere for at least 3 days was apparently essential to these plants in alleviating internal water deficit sufficiently to permit root establishment.

All adventitious roots, having become established in the soil to a depth of 50 mm., seemed equally capable of survival on all plants remaining alive through the drought period, irrespective of position or age. Their growth was undoubtedly limited by the size of the container, and it is probable that fewer plants with these roots would have died on the longer drought periods if a greater volume of soil had been available for them. In nature, drought survival of many grasses in arid regions must be dependent, during drought dormancy, upon two major factors: first, the meager supplies of water furnished to the basal stem meristems by those roots penetrating into soil layers in which water content is not yet reduced to complete nonavailability; and second, any physiological adaptations, involving bound

water or related phenomena, in the meristems themselves. The zones of possible absorption in such roots might warrant investigation, in view of apparent maturation almost to the tip during drought, reported in this study.

Discussion

In considering the effects of drought on correlations within the plant, as illustrated by its differing effects on initiation and growth of stems and leaves in contrast with roots, the positive correlation in the growth of the former with the amount of water supplied over the 4 months of the experiment seems somewhat remarkable. If considered from the standpoint of averages, it suggests that—aside from those plants which failed to survive the drought period—the differing number, position, and time of establishment of adventitious roots had little effect in producing differential survival and growth of the various parts of the shoot system. Correlations were practically lacking also on an individual plant basis. If progressive determinations of absorbing surface were practical, or if it had been possible to obtain accurate dry-weight data on roots, correlations of these values with the various criteria of shoot growth might have been positive. Even this, however, would not explain the inference that in plants, up to 4 months old, water transmitted from the various adventitious roots—regardless of position—and from the primary root system apparently is equally available to all shoot-growing tips. In individual plants there was some suggestion that any exceptionally vigorous tillers had established adventitious roots, either before the drought period or soon after the resumption of watering. A cause and effect relation might be implied, which undoubtedly becomes operative in older plants.

The bearing of this study upon possible reasons for the early increase of *Bouteloua curtipendula* in the prairie during the years of drought is somewhat perplexing. WEAVER and ALBERTSON (10) attributed this increase partly to the prolific seeding habit of the species, suggesting that seedlings played an important role in the increase. Without causal data on mortality and survival of seedlings of different ages in this region during this period, correlations with the results of this study are impossible. But the latter indicates that such survival and increase can be considered remarkable. Aside from the rapid and deep penetration of its single primary root, this species shows no morphological equipment during its early seedling life which would indicate high drought resistance or success under arid conditions. The lack of more than one seminal root, the production of the first adventitious root when 2 or more weeks old, the slow rate of initiation of additional ones, the period of soil wetness required for their successful establishment, the apparent inability of the first-formed primordia to remain functional through more than 2–3 weeks of internal water deficit, and the establishment of the crown nodes at the surface of the ground in any depth of planting so far used by the

writer—these would all seem to be disadvantageous. Against this may be noted, at least for older plants, the amount of branching and rate of penetration of established adventitious roots, the stimulation in root-primordium production when adventitious roots have failed in establishment, and the apparent equal availability of a limited water supply to all stem growing points located near the ground, together with their protection against desiccation. These growing points are the meristems which eventually give rise to new roots as well as leaves. It may well be that drought resistance in perennial grasses rests partly upon the success of the stem meristems in obtaining their full share of a limited supply of water. In this species they were obviously more efficient in this respect than were those of the roots, which renewed growth very slowly upon resumption of favorable conditions, in contrast with the stem meristems. In this connection, it is well to bear in mind that the pathway and mechanism of transmission of water to stem growing points when drought-dormant grasses resume growth has never been completely determined (8).

Summary

1. Seedlings of *Bouteloua curtipendula* in 2-gallon glazed crocks, grown under wet to dry conditions as regulated by watering at intervals of 3, 6, 12, or 20 days, were subjected to soil-drought periods from 48 to 88 days in length. Plants in the first three series were subjected to drought when 18, 30, and 42 days old, and in the last at 26 and 46 days. Watering was resumed in some pots of each series after 48 days of drought, at intervals of 3 and 12 days. Others were left on drought until approximately 110 days old and were then watered amply to test survival. All plants were harvested at 119 days, except those harvested at the beginning, at the close, and 3 days after the close of the 48-day drought.

2. The few seedlings which failed to survive the 48-day drought, and many of the larger number failing to survive for longer periods, had failed in establishment of adventitious roots, either because of having been placed on drought prior to, or just at, the time of initiation of such roots, or because infrequent watering prevented their establishment. Such establishment seemed to be dependent upon 3 consecutive days of soil-surface wetness. A number of small plants, however, survived 48 days of drought with no established adventitious roots.

3. All stem meristems nearest the ground, and the young foliage surrounding them, were equally and most resistant to the effects of internal water deficit, resuming growth rapidly after watering. Older foliage died during the 48-day drought period. Initiated adventitious root primordia, buried in stem tissues, either died or failed to grow effectively after subjection to 2-3 weeks of water deficit. Many root tips, established in the soil, had apparently survived but resumed growth very slowly after drought, in contrast to stem meristems.

4. There was little differential effect of drought at different ages on the growth produced by all stem meristems which survived. At 119 days, numerous growth measurements were correlated closely with total amount of water supplied during the entire experiment. In general, size was reduced by drought to a greater degree than were numbers of organs differentiated, at least in the 48-day period, as compared with continuously watered controls.

5. Drought, when it prevented establishment of adventitious roots, tended to stimulate the initiation of root primordia on the various axes, both by acceleration of rate of initiation and by increase in total numbers produced on an axis, after watering was resumed.

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LITERATURE CITED

1. AAMODT, O. S., and JOHNSTON, W. H., Studies on drought resistance in spring wheat. *Canad. Jour. Res. (C)* 14:122-152. 1936.
2. BAILEY, L. F., Some water relations of three western grasses. I. The transpiration ratio. II. Drought resistance. III. Root development. *Amer. Jour. Bot.* 27:122-135. 1940.
3. GLENDENING, G. E., Development of seedlings of *Heteropogon contortus* as related to soil moisture and competition. *BOT. GAZ.* 102:684-698. 1941.
4. GOURLEY, J. H., and HOWLETT, F. S., Modern fruit production. New York. 1941.
5. LLOYD, F. E., Environmental changes and their effect upon boll shedding in cotton. *Ann. New York Acad. Sci.* 29:1-131. 1920.
6. MARTIN, J. N., and HERSHEY, A. L., The ontogeny of the maize plant—the early differentiation of stem and root structures and their morphological relationships. Symposia commemorating six decades of the modern era in botanical science 1:275-289. Reprinted from *Iowa State Coll. Jour. Sci.* 9. 1935.
7. MAXIMOV, N. A., The plant in relation to water. London, England, 1929. (Eng. transl. by R. H. YAPP.)
8. NEDROW, W. W., Studies on the ecology of roots. *Ecology* 18:27-52. 1937.
9. OLMSTED, C. E., Growth and development in range grasses. I. Early development of *Bouteloua curtipendula* in relation to water supply. *BOT. GAZ.* 102:499-519. 1941.
10. WEAVER, J. E., and ALBERTSON, F. W., Major changes in grassland as a result of continued drought. *BOT. GAZ.* 100:576-591. 1939.
11. WEAVER, J. E., STODDART, L. A., and NOLL, WM., Response of the prairie to the great drought of 1934. *Ecology* 16:612-629. 1935.

QUANTITATIVE STUDY OF ETHYLENE PRODUCTION IN RELATION TO RESPIRATION OF PEARS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 538

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(WITH SEVEN FIGURES)

Introduction

Recent investigations have shown that volatile substances other than carbon dioxide are produced by apples (2, 4), pears (8), and certain other fruits (15). These emanations, although present in minute amounts, have been found to influence the respiration and other metabolic activities associated with ripening processes. That the active principle concerned with producing these effects is ethylene has been established definitely (3, 9, 20). In view of these findings, the factors associated with the formation of ethylene in fruits and its production in relation to other phases of metabolism have been studied.

GANE (4) has measured the quantity of combustible emanations from Marie Louise pears and found that the amounts produced increased rapidly at the climacteric. With apples he found some indication that the more rapidly respiring varieties were in general more active in the production of volatiles. KIDD and co-workers (12) have estimated by combustion the total carbon-containing substances other than CO₂ which are evolved during the ripening of Conference pears stored at 10° C. and found that the maximum rate of production of these emanations corresponded to the maximum of the initial rise in respiratory activity. They did not find that this increase was due to an increase in the rates of escape of alcohol and acetaldehyde. GERHARDT and EZELL (5), using concentrated sulphuric acid as an absorbent, studied the emanation of volatiles in relation to respiration of Bartlett pears during ripening at 65° F. and found that the climacteric for respiration preceded that for total volatiles by approximately 7 days. They state, however, that a considerable portion of the emanations from fruits of this variety may result from the gross liberation of acetaldehyde from the tissues.

The development of microchemical methods (1, 16, 20) for the determination of ethylene has made it possible to isolate ethylene from all other volatile compounds produced by fruits and to estimate it separately. NELSON (16), by use of a potassium-permanganate oxidation method, has made a quantitative study of ethylene production by apples and bananas and has shown that an apparent

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relationship exists between the keeping qualities of apples and the amounts of ethylene which they produce.

The present investigation was undertaken for the purpose of making a quantitative study of ethylene production in relation to respiration of pears. The emanation of this gas during ripening in air at 20° C.—and under conditions by which respiration was altered by changes in temperature and oxygen tension—has been studied.

Material and methods

SOURCE AND HANDLING OF FRUIT.—The three varieties of pears—Bartlett, Doyenne du Comice, and Beurre d'Anjou—used in this investigation were obtained from the Medford, Oregon, district. The fruit was picked at the regular commercial picking date for the variety, packed in oil wraps, and stored at 0° C. (32° F.). During the fall of 1939, the fruit was shipped under refrigeration to the University of Chicago, where the experimental work for the first season was carried out. The methods used in handling the fruit after removal from cold storage varied with different lots, as later specified.

ANALYTICAL METHODS.—The method for determining the CO₂ and O₂ of respiration was that of HALLER and ROSE (7) as modified by MASURE (14). Approximately 2.5 kilograms of fruit were inclosed for 15–20 hours each day in large desiccators. Oxygen was supplied from calibrated 2.5-liter bottles connected to the desiccators by means of 1-mm. capillary tubing. The oxygen inlets were fitted with mercury valves to prevent backward diffusion of air and the evolved ethylene into the oxygen-supply bottle. The fruit was held at 20° C. (69° F.) during ripening, except for certain experiments as later specified. The use of this method (in which the fruit is kept in a confined air space) made it possible to determine CO₂, O₂, and ethylene on the same fruit sample. In this procedure the evolved ethylene accumulates in the desiccators during confinement of the fruit, and this fact must be taken into consideration in interpreting any data obtained. The recent work of KIDD and WEST (11) and HULME (10), however, has shown that mass lots of fruit cannot be kept free from ethylene, even where ventilated, so that it tends to exert an effect under any method used unless individual fruits are kept isolated from one another.

Ethylene was estimated by the micro-bromination method previously described (1, 9). In order to utilize a larger gas sample for analyses than originally specified, the following modified procedure was used. After an aliquot of the potassium-hydroxide solution had been withdrawn for titration, the desiccator was connected to a 220-ml. gas-sampling bulb and a sample of air for ethylene analysis withdrawn. Then 0.2 ml. of 28 per cent ammonium-hydroxide solution was introduced from a pipette connected to the side arm of a three-way stopcock on the lower end of the sampling bulb by means of a short piece of rubber tubing. The

leveling bulb was then raised and the ammonia—together with approximately 10 ml. of mercury—was allowed to mix with the gas sample. The bulb was then shaken and allowed to stand for 15 minutes to insure complete absorption of any acetaldehyde present. A portion of the gas sample was finally passed slowly through the purification unit, as previously described, and collected over mercury in a 100-ml. gas burette.

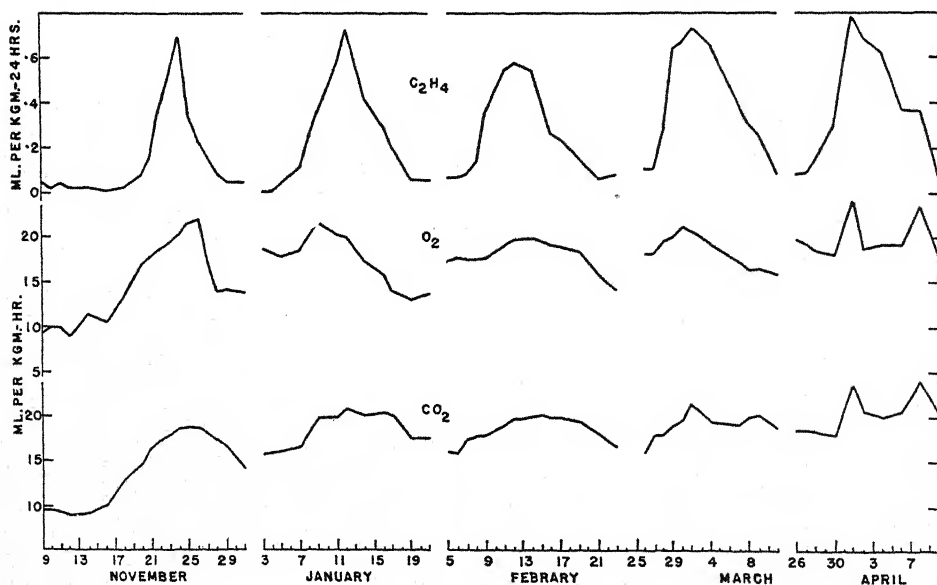
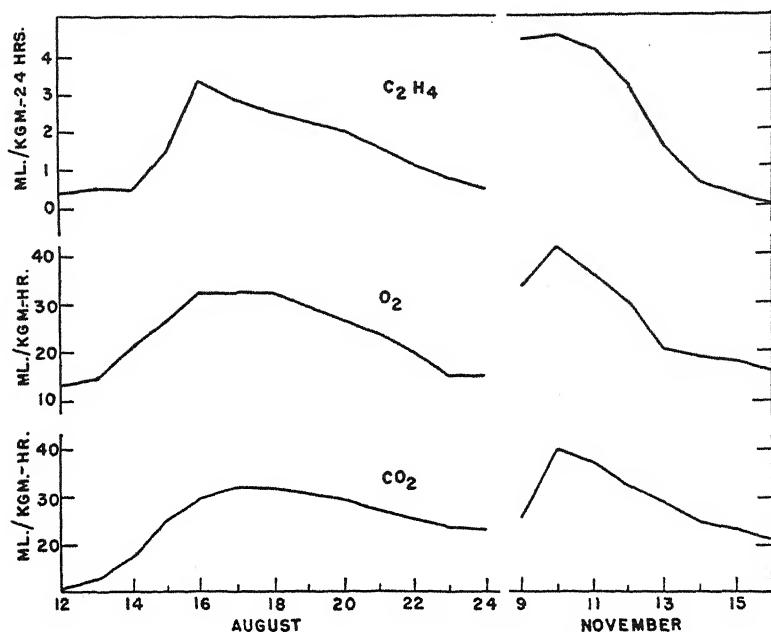
To a 125-ml. reaction flask, identical in design except for size to the one previously described, was added 10 ml. of 0.0025 N potassium bromate and 1 ml. of 6 N sulphuric acid. The flask was evacuated to approximately 27.5 inches of mercury, connected to the burette, and the purified ethylene sample then drawn in by opening the stopcocks and raising the leveling bulb. Since mercury brominates readily, care was taken to prevent its introduction into the flask. To guard against this, a short piece of ordinary tobacco pipe-cleaner was inserted into the capillary outlet of the gas burette. After introducing the gas sample, 2 ml. of N/10 potassium bromide was added from a pipette connected by rubber tubing to the reaction flask. The flask was then shaken for 15 minutes on a horizontal mechanical shaker operating at approximately 100 oscillations per minute. Two ml. of 2.5 per cent potassium iodide was then introduced. After vigorously shaking the solution, a wash bottle was connected to the flask and the neck washed down by drawing in water by means of the residual vacuum. To insure complete absorption of the free bromine, the flask was allowed to stand for 2 minutes, with occasional gentle rotation. The stopcock was then removed, rinsed down carefully with distilled water, and the solution titrated with 0.0025 N sodium thiosulphate from a 10×0.02 micro-burette. Duplicate determinations were made on each gas sample.

Experimental results

RELATION OF ETHYLENE PRODUCTION TO RESPIRATION DURING RIPENING AT 20° C. BEFORE AND AFTER STORAGE AT 0° C.

In order to follow the trend of ethylene production in relation to respiration during ripening, samples of fruit were withdrawn at different intervals throughout the storage period and ripened at 20° C. Two varieties of pears were used for these studies. One lot of Bartletts was ripened immediately after picking and the second after being held in storage for 3 months. Five lots of Anjous were withdrawn at approximately monthly intervals, beginning on the third month. The CO_2 and O_2 of respiration, as well as the amounts of ethylene evolved, were determined daily during ripening.

The Bartlett pears ripened immediately after picking showed a low rate of respiration as well as emanation of ethylene at the beginning of the experiment (fig. 1). Both processes began to increase at the same time, but the peak in ethylene production was reached one day earlier than the maximum for respiration.



FIGS. 1, 2.—Fig. 1 (top), ethylene production and respiration of Bartlett pears during ripening before and after storage at $0^{\circ}C$. Fig. 2 (bottom), ethylene production and respiration of Anjou pears during ripening after progressive storage periods.

During the post-climacteric period, ethylene production and oxygen consumption fell off rapidly, while the production of CO_2 showed a much slower rate of decline. The ratio CO_2/O_2 changed markedly during the course of ripening. At the beginning the value was approximately 0.8. At the climacteric, when the fruit was fully ripe, it had increased to unity but rose to 1.4 at the termination of the experiment. These wide changes in the respiratory quotient indicate that qualitative as well as quantitative changes occur in respiration during ripening.

The sample of Bartlett pears held for 3 months in cold storage was still fairly firm but had begun to show a yellow color when withdrawn for ripening. The fruit became fully ripe after being held for 4 days at 20°C . The curves for respiration and ethylene production for this lot differ in some respects from those of the fruit ripened immediately after picking. The peak in respiration was reached on the second day as compared with the fifth day for the first lot. Ethylene production was at a maximum on the second day and declined steadily thereafter, finally falling below the amount that could be quantitatively measured.

The data for the Anjou pears are presented in figure 2. In the first lot ripened, ethylene production remained at a very low level (0.009–0.03 ml. per kg./24 hour) for 7 days before a steady rise began. During the following 6 days there was a twenty-three-fold increase, followed by a sharp decline when the fruit became overripe. The curves for samples of fruit ripened at later dates are similar, except that the increase in ethylene production began immediately on transfer to the higher temperature.

Certain differences were observed in the trend of respiration during ripening of fruit withdrawn at different dates during the storage period. In the first sample, CO_2 output and O_2 intake remained at a low level for 7 days and then approximately doubled during the following 6 days. The peak in respiration coincided very closely with the peak in ethylene production. For the samples of fruit withdrawn at later dates there was a steady increase in the initial rates of CO_2 production and O_2 consumption, so that the sharp incline characteristic of the climacteric rise was of shorter duration. In the last sample of fruit ripened, a secondary increase in respiration occurred during the post-climacteric period. No increase in rate of ethylene production occurred during this final rise in CO_2 output and O_2 intake. KIDD and co-workers (12) have reported that total combustible volatiles of Conference pears do not increase during the secondary rise in respiration.

Table 1 presents the initial and maximum rates of evolution of CO_2 and ethylene for each lot of fruit ripened at the different dates during the storage period. The relative increases, calculated as maximum rate/initial rate, are also given.

The data of table 1 indicate certain well-defined trends for respiration and for ethylene production during storage and ripening. There is a progressive increase

in the initial rate of respiration for each lot of fruit when removed from cold storage to warm temperature. In the Bartletts this increase was from 11.61 to 25.90 ml. per kg.-hour, and in the Anjous from 9.45 to 18.23 ml. per kg.-hour. The initial rates of ethylene production likewise showed distinct indication of increasing. In Bartletts the range was from 0.49 to 4.39 ml. per kg.-day and in Anjous from 0.009 to 0.12 ml. per kg.-day. As shown in the table, the relative increases during the climacteric are much greater for ethylene production than for respiration. This may indicate that the two processes are not directly related. Additional evi-

TABLE 1
INITIAL AND MAXIMUM RATES OF CARBON DIOXIDE AND ETHYLENE PRODUCTION
AT 20° C. AFTER DIFFERENT PERIODS OF STORAGE AT 0° C.

MONTHS STORED	RATE OF RESPIRATION (ML. PER KG.-HOUR)			RATE OF ETHYLENE PRODUCTION (ML. PER KG.-DAY)		
	INITIAL	MAXIMUM	INCREASE	INITIAL	MAXIMUM	INCREASE
BARTLETT						
0.....	11.61	33.03	2.85	0.490	3.25	6.63
3.....	25.90	39.89	1.54	4.390	4.48	1.01
ANJOU						
2.....	9.45	18.79	1.99	0.009	0.700	78.80
4.....	15.76	20.72	1.31	0.010	0.726	72.60
5.....	16.03	19.89	1.24	0.065	0.570	8.77
6.....	15.60	21.00	1.35	0.105	0.761	7.25
7.....	18.23	23.27	1.28	0.120	0.782	6.52

dence in favor of this view is shown by the wide varietal differences in respiratory activity and ethylene production. The maximum rate of respiration for Bartlett pears is approximately double the rate for Anjous, yet the maximum rate of ethylene production is six or seven times greater in Bartletts than in Anjous.

The production of ethylene in relation to respiration during ripening of pears is distinctly different from that for apples and bananas as reported by NELSON (17, 19). He found that during the climacteric for these fruits, ethylene production decreased but increased again in the post-climacteric stage. Thus there was an inverse correlation between the emanation of ethylene and the production of CO₂. He suggested that ethylene was "consumed" during respiration and presented experimental evidence to that effect (18). If ethylene is metabolized during respiration of pears, the highest rate must occur when respiratory activity is low,

since the highest rates of ethylene production were found to coincide with the highest rates of respiration. No experimental evidence could be obtained, however, that ethylene is oxidized or otherwise metabolized by pears when respiratory activity is either high or low. Pure ethylene in small amounts was added to a desiccator containing Anjou pears in all stages of ripeness. There was an initial loss during the first 2 hours, probably due to adsorption of the gas on the glass walls and diffusion into the fruit; no further loss could be detected. The apparent discrepancies found between the emanation of ethylene and the respiration of pears cannot be explained on the assumption that ethylene is metabolized by the fruit.

No explanation is apparent at the present time for the differences in the relation of ethylene production to respiration of pears and apples. The time relation for the occurrence of the climacteric is different for the two fruits, and this fact may have some bearing on the discrepancy found. In apples (as well as in bananas) the climacteric precedes actual ripening of the fruit. In pears, however, ripening begins with the climacteric rise, and at the initial respiratory maximum the fruit is fully ripe.

PRODUCTION OF ETHYLENE IN RELATION TO RESPIRATION UNDER CONDITIONS OF ALTERED TEMPERATURES AND OXYGEN CONCENTRATIONS

The data presented in the previous section have shown that ethylene production and respiration do not follow precisely parallel courses during ripening at 20° C. Since respiration can be altered by several experimental treatments, the production of ethylene under similar treatments was investigated.

The production of ethylene when fruit is maintained under anaerobic conditions was investigated first. For this experiment, three lots of Bartlett pears which had been in storage for 3 months were used. The fruit was kept in air for the first day. On the second day the air in the several desiccators was displaced with nitrogen, hydrogen, and helium, respectively. On the third day all lots were again transferred to air. Determinations of CO₂ and ethylene were made daily.

It is apparent from the results obtained (fig. 3) that the emanation of ethylene is not associated with the production of CO₂ under anaerobic conditions. Upon transfer of the fruit to oxygen-free atmospheres, ethylene production was either greatly retarded or totally inhibited. When transferred to air, emanation again took place. The production of CO₂ under aerobic and anaerobic conditions, however, changed very little.

This experiment was repeated the following season on newly-picked Bartlett pears. One lot of fruit was kept for 3 days in air, transferred to nitrogen on the fourth day, and then maintained in air for an additional 5 days. A second lot was kept continuously in air. The results are shown in figure 4.

The sample of fruit confined continuously in air showed the usual climacteric rise and increase in ethylene production followed by the sharp decline shown previously for pre-climacteric pears. The behavior of the treated lot, however, was decidedly different. On transferring the fruit from air to nitrogen, the rate of ethylene production declined below the amount quantitatively determinable. Respiration as measured by CO_2 showed very little change. On transfer from nitrogen to air, ethylene production increased to slightly above the maximum rate for the control fruit, and thereafter ethylene production in both lots declined at approximately the same rate. The production of CO_2 , on the other hand, declined

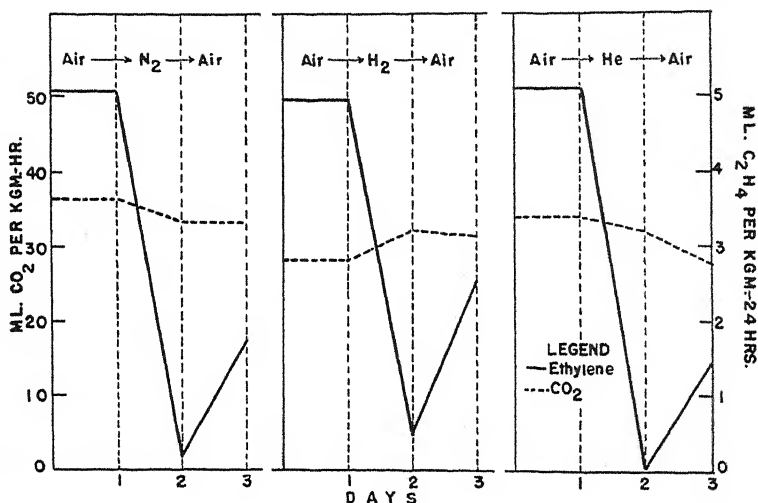


FIG. 3.—Comparative production of ethylene by Bartlett pears under aerobic and anaerobic conditions.

the first day following nitrogen treatment, then increased slightly before showing the senescent decline. The striking feature shown in this experiment is that subjecting the fruit to anaerobic conditions for 1 day prevented completion of the climacteric rise. In spite of this fact, ethylene production rose from zero to slightly above the maximum rate shown by the fruit held continuously in air. It has been possible, therefore, experimentally to decrease the magnitude of the climacteric without altering the usual trend of ethylene production.

The effect of temperature on respiration and ethylene production was also investigated. For these experiments Comice pears which had been kept in storage for 2 months were used. The fruit was brought directly from 0° C. storage, carefully sorted into five uniform lots, and then placed in constant temperature cabinets maintained at 0°, 10°, 20°, 30°, and 40° C., respectively. After the temperature of the fruit had reached the respective air temperature, it was placed in

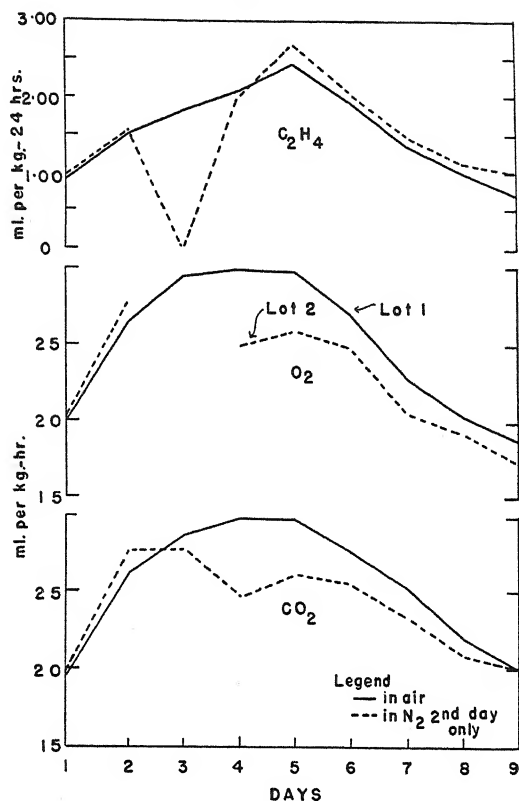


FIG. 4.—Effect of nitrogen storage of Bartlett pears on subsequent production of ethylene and respiration in air. Lot 1 held continuously in air at 20° C. Lot 2 transferred to nitrogen on second day but held continuously in air thereafter.

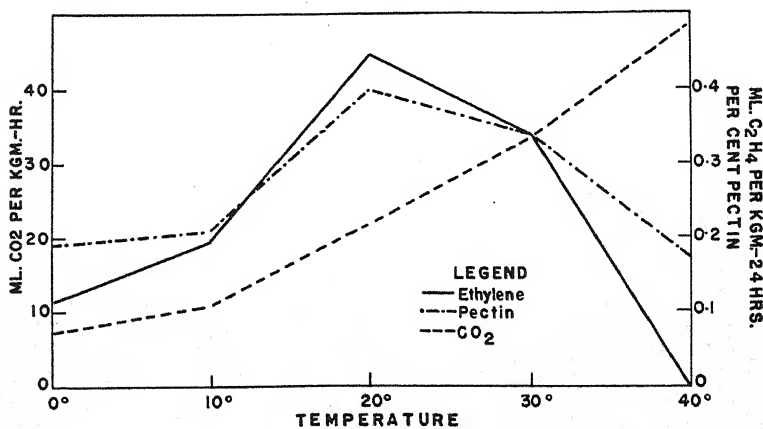


FIG. 5.—Effect of temperature on ethylene production, respiration, and pectin formation in Comice pears.

desiccators and supplied with oxygen as previously described. Determinations of CO_2 and ethylene were made after 24 hours. One-half of each lot was then used for the determination of the internal concentration of CO_2 and O_2 . The remainder of the fruit was kept for an additional 5 days for soluble pectin analyses.

That changes in temperature do not affect ethylene production and respiration to the same degree is apparent from the data obtained (fig. 5). Between 0° and 20° , ethylene and CO_2 production both increased, the former reaching a maximum at the higher temperature. From 20° to 40° , CO_2 production continued to increase steadily but the rate of ethylene evolution declined sharply, reaching a zero value at 40° . The rate of soluble pectin formation also increased up to 20° , but declined thereafter. It is known from previous experiments (21) that the ripening of pears

TABLE 2
EFFECT OF TEMPERATURE ON CO_2 AND O_2
CONTENT OF COMICE PEARS

TEMPERATURE ($^\circ\text{C}.$)	PERCENTAGE CO_2	PERCENTAGE O_2
0.....	9.99	26.61
10.....	18.56	16.19
20.....	23.33	12.62
30.....	30.50	6.57
40.....	27.50	4.51

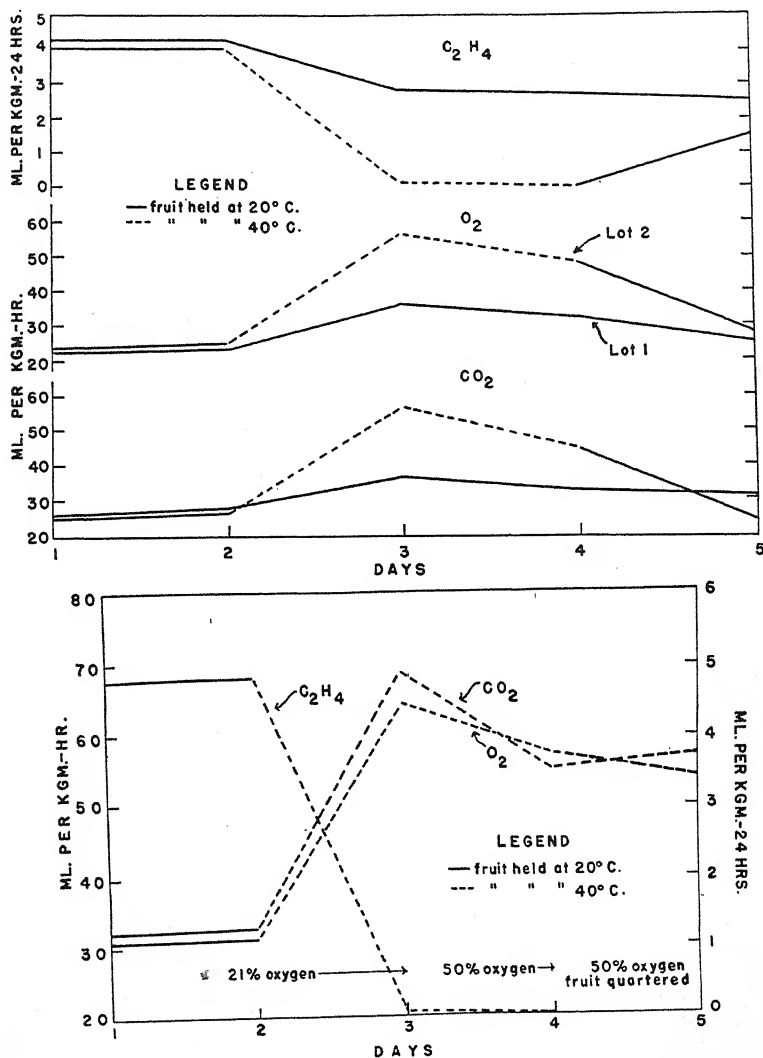
is retarded by high temperatures, but the significant fact shown in the present experiment is that ethylene production is similarly retarded.

The data presented in table 2 show that with increase in temperature there is an increase of CO_2 and a decrease of O_2 in the fruit tissues. From these data it could be assumed that at high temperatures, oxygen became a limiting factor so that no ethylene was produced. To investigate this possibility, the following experiments with Bartlett pears were carried out. One lot of fruit was maintained continuously at 20° during ripening. A second lot was kept for 2 days at 20° , then transferred to 40° for 2 days, and finally returned to 20° for 1 day. Oxygen consumption in addition to ethylene and CO_2 production was measured daily.

As shown in figure 6, ethylene production declined from approximately 4 ml. per kg.-day to zero on transfer of the fruit to the higher temperature. In contrast, respiration as measured by either CO_2 or O_2 greatly increased, the RQ being slightly greater than unity at 40°C . When transferred to 20° , emanation again occurred, showing that destruction of the ethylene precursor or enzyme system had not taken place.

In a second experiment the effect of increasing the concentration of oxygen in fruit held at 40° was investigated. The first day the fruit was confined in air.

The second day the same oxygen concentration was maintained, but the temperature was raised to 40° . On the following day the concentration of oxygen was in-



FIGS. 6, 7.—Fig. 6 (top), effect of temperature on ethylene production and respiration of Bartlett pears. Fig. 7 (bottom), effect of increasing oxygen tension of Bartlett pears held at $40^{\circ} C.$ on ethylene production and respiration.

creased to 50 per cent. On the final day at the higher temperature the fruit was cut into quarters but retained in 50 per cent oxygen.

The data (fig. 7) show that increasing the oxygen concentration to 50 per cent

or increasing the absorptive surface by quartering the fruit had no effect on ethylene production at 40°. There was a small amount of ethylene found the first day on transfer to high temperature, but the production dropped to zero thereafter. Oxygen consumption as well as CO₂ production increased with rise in temperature, clearly demonstrating that aerobic respiration was taking place. In spite of this fact, no ethylene was produced. It could be assumed that probably the high content of CO₂ in the tissues inhibited ethylene production, or that ethylene was metabolized by the fruit when kept at high temperatures. Both these possibilities were investigated but with negative results. Ethylene production was found to be retarded but not inhibited in a mixture of 33 per cent CO₂ and 21 per cent O₂. Nor could any loss of ethylene be detected at 40° when small amounts of the gas were added to the desiccator according to the method used by NELSON (18).

Analysis of the gases extracted from pears kept at 40° showed no ethylene present. The addition of synthetic ethylene to fruit maintained at high temperatures, however, did not have any effect on ripening, the fruit remaining hard and green after 6 days' treatment. These data show that the mere presence of ethylene in the tissues is not sufficient to bring about ripening of the fruit under these conditions. Apparently the specific reaction from which ethylene is formed as a product must take place when the fruit ripens.

EFFECT OF H₂S AND HCN AS RESPIRATORY INHIBITORS

The data presented in the previous section strongly indicate that ethylene production is associated with a special oxidative system which is inhibited by high temperature. The amount of oxygen required for the functioning of this system apparently is smaller than could be detected or accurately measured by the methods used. Since certain of the oxidative enzymes are known to be inhibited by such substances as H₂S and HCN, response of the specific enzymes associated with respiration and ethylene production might differ in relation to these reagents. To investigate this possibility, Anjou pears in the post-climacteric stage were treated with H₂S and HCN in concentrations ranging from 0.1 to 4 per cent. The results obtained from these experiments have been inconclusive. Neither ethylene production nor oxygen consumption was affected by these reagents at the low concentrations found to be effective on other kinds of plant structures. At higher concentrations than those generally used, both oxygen uptake and ethylene production were partially inhibited. In one experiment a concentration of 4 per cent H₂S completely inhibited ethylene production and greatly retarded oxygen consumption. The fruit, however, showed severe injury after 2 days' exposure and did not ripen when treatment was terminated. The only conclusion that can be made at the present time from these data is that fruit in the post-

climacteric stage is not sensitive to the low concentrations of H_2S or HCN found to retard respiration in other kinds of tissues. It is possible that the reaction of enzymes to these substances is not the same at all stages of development of the fruit. MARSH and GODDARD (13) found the respiration of young carrot leaves to be inhibited by NaN_3 , HCN , and CO , but when the leaves became older the respiration was not affected by any of these poisons.

Discussion

The quantitative data obtained during the course of this investigation show that the production of ethylene is a definite part of the metabolism of mature pears. If the formation of ethylene resulted from a chance reaction, then it would be expected that emanation of the gas would be erratic and follow no well-defined trend. This has not been found to be the case. Each sample of fruit ripened at 20°C . showed the same general trend of production—an initial increase during the climacteric rise, the attainment of a maximum rate, and a decline during the senescent period. Moreover, each of the varieties studied over an extended period was found to have a definite, characteristic maximum rate of production, and this value varied but little among the different lots of fruit ripened. It may be concluded that the formation of ethylene is an integral part of the metabolic activities of mature pears. This fact is significant in that it forms the basis for the consideration of ethylene production in relation to other phases of fruit metabolism.

Determinations of the rate of ethylene production and of respiration were carried out simultaneously on the same samples of fruit. The results show that the two processes follow somewhat the same general trend during ripening, but no indications that ethylene production is directly related to respiration were found. In the first place, the relative increase in ethylene production during the climacteric rise is out of all proportion to the increase in the rate of respiration as measured by either CO_2 or O_2 . Thus, respiratory activity approximately doubles during this initial stage of ripening, while ethylene production increases seven- to eighty-fold, depending upon the variety and length of time it has been held in cold storage prior to ripening. During the post-climacteric stage, the emanation of ethylene declines rapidly, but respiration, especially as measured by CO_2 , declines at a much slower rate. Moreover, the last sample of Anjou pears ripened late in the storage season showed a secondary rise in respiration during senescence, but this was not accompanied by a similar rise in ethylene production. Additional evidence that the production of ethylene and respiration are not necessarily directly related is shown by the wide differences in the ratio $\text{CO}_2/\text{C}_2\text{H}_4$ for the two varieties of fruit studied. During the climacteric, Bartlett pears respire at approximately double the rate for Anjou, yet the rate of ethylene production is six or seven times greater in the former variety.

When pears were subjected to experimental alterations in oxygen tension and temperature, ethylene production and respiration were found to become even more widely separated. In oxygen-free atmospheres the formation of ethylene was either greatly retarded or entirely inhibited. In contrast, the rate of CO_2 evolution was changed but little by this treatment, showing that the formation of ethylene is not correlated with CO_2 production under anaerobic conditions.

Further evidence that ethylene production and respiration are not directly related is shown by their difference in response to high temperatures. From 0° to 20° C., the Q_{10} for the two processes was found to be approximately the same. At higher temperatures, however, the responses were entirely different. Respiratory activity continued to rise with increase in temperature beyond 20° . On the contrary, ethylene production declined rapidly, and at 40° C. no emanation could be quantitatively detected. That oxygen supply is not a limiting factor under these conditions has been shown. Thus, even though the rate of aerobic respiration was greatly accelerated at 40° , the production of ethylene was entirely inhibited, apparently by the inhibition of the reactions associated with its formation.

Since there appears to be no positive correlation between ethylene production and respiration under either anaerobic or aerobic conditions, it seems necessary to postulate that the formation of ethylene is not directly associated with that phase of respiration responsible for the major portion of the CO_2 produced and O_2 consumed. It can by no means be assumed, however, that the method of formation of ethylene is to be considered as entirely separate and distinct from the respiratory mechanism in fruits. Respiration in fruits, as in certain other plant structures (6, 22), may consist of several distinct but interrelated systems—some requiring oxygen, others not; but each contributes to the total amount of CO_2 being measured. According to this concept, the total amounts of O_2 consumed or CO_2 produced by the fruit will not necessarily be correlated with the activity of each individual system of the respiratory complex.

Summary

1. A quantitative study of ethylene production in relation to the respiration of pears has been made. The emanation of ethylene in air at 20° C., as well as at higher and lower temperatures in modified atmospheres, has been studied.
2. In fruit in air at 20° C. the rate of ethylene production increases during the climacteric rise in respiration, reaches a peak at the respiratory climax, then declines during the post-climacteric period. During the climacteric rise, ethylene production increases seven- to eighty-fold, while rate of respiration approximately doubles.
3. Each variety was found to have a characteristic maximum rate of production. The maximum rate for Bartlett, a variety which maintains its capacity to

ripen for only a short period of time when kept at a storage temperature of 0° C., is 3.25–4.48 ml. per kg.-day. The maximum rate for Anjou, a variety which maintains its capacity to ripen for a long period of time when kept at cold storage temperatures, is 0.57–0.78 ml. per kg.-day. The maximum rate of respiration for Bartlett is approximately double that for Anjou.

4. Under anaerobic conditions, the production of ethylene is either greatly retarded or entirely inhibited. In the fruits used for these experiments, there was found but little difference in the production of CO_2 under aerobic and anaerobic conditions.

5. The maximum rate of ethylene production occurs at 20° C. At higher temperatures production decreases and is totally inhibited at 40° C. Respiratory activity as measured by either CO_2 production or O_2 consumption is greatly accelerated between 20° and 40° C.

The writer expresses his thanks to Dr. C. A. SHULL and others of the botany department of the University of Chicago for their assistance, and to Professor F. C. REIMER, Superintendent of the Southern Oregon Experiment Station, for supplying the fruit used in this investigation.

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LITERATURE CITED

1. CHRISTENSEN, B. E., HANSEN, ELMER, and CHELDELIN, V. H., Determination of ethylene in the internal atmosphere of plant tissues. *Ind. Eng. Chem. Anal. Ed.* 11:114–116. 1939.
2. ELMER, O. H., Growth inhibition of potato sprouts by volatile products of apples. *Science* 75:193. 1932.
3. GANE, R., The formation of ethylene by plant tissues and its significance in the ripening of fruits. *Jour. Pomol. Hort. Sci.* 13:351–358. 1935.
4. ———, Volatile products of fruits. *Great Britain Dept. Sci. and Ind. Res. Food Invest. Bd. Rept.* Pp. 127–129. 1935.
5. GERHARDT, FISK, and EZELL, B. D., Respiration and emanation of volatiles from Bartlett pears as influenced by ripening and storage. *Proc. Amer. Soc. Hort. Sci.* 36:423–426. 1938.
6. GREGORY, F. G., and SEN, P. K., Physiological studies in plant nutrition. *Ann. Bot. N.S.* 1:521–561. 1937.
7. HALLER, M. H., and ROSE, D. H., Apparatus for determination of CO_2 and O_2 of respiration. *Science* 75:439–440. 1932.
8. HANSEN, ELMER, and HARTMAN, HENRY, The occurrence in pears of metabolic gases other than carbon dioxide. *Ore. Exp. Sta. Bull.* 342. 1935.
9. HANSEN, ELMER, and CHRISTENSEN, B. E., Chemical determination of ethylene in the emanations from apples and pears. *BOT. GAZ.* 101:403–409. 1939.

10. HULME, A. C., An apparatus for measuring the output of carbon dioxide by a sample of 2-4 kg. of apple fruits. Great Britain Dept. Sci. and Ind. Res. Food Invest. Bd. Rept. Pp. 133-136. 1937.
11. KIDD, F., and WEST, C., The influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Great Britain Dept. Sci. and Ind. Res. Food Invest. Bd. Rept. Pp. 51-57. 1933.
12. KIDD, F., GRIFFITHS, D. G., and POTTER, N. A., An investigation of the changes in chemical composition and respiration during ripening and storage of Conference pears. Ann. Bot. 4:1-30. 1940.
13. MARSH, P. B., and GODDARD, D. R., Respiration and fermentation in the carrot, *Daucus carota*. I. Respiration. Amer. Jour. Bot. 26:724-728. 1940.
14. MASURE, M. P., Some comparisons of methods of measuring fruit respiration. Proc. Amer. Soc. Hort. Sci. 36:223-229. 1938.
15. MILLER, E. V., WINSTON, J. R., and FISHER, D. F., Production of epinasty by emanations from normal and decaying citrus and from *Penicillium digitatum*. Jour. Agr. Res. 60:269-277. 1940.
16. NELSON, R. C., The quantity of ethylene present in apples. Plant Physiol. 12:1004-1005. 1937.
17. ———, Physiology of ethylene production, use and reaction in plants. Proc. Minn. Acad. Sci. 6:37-41. 1938.
18. ———, The production and consumption of ethylene by ethylene-treated bananas. Plant Physiol. 14:817-822. 1939.
19. ———, Quantitative study of the production of ethylene by ripening McIntosh apples. Plant Physiol. 15:150-151. 1940.
20. NIEDERL, J. B., and BRENNER, M. W., The identification and estimation of ethylene in the volatile products of ripening bananas. Amer. Jour. Bot. 25:357-361. 1938.
21. OVERHOLSER, E. L., and TAYLOR, R. H., Ripening of pears and apples as modified by extreme temperatures. BOT. GAZ. 69:273-296. 1920.
22. STUART, F. C., STOUT, P. R., and PRESTON, C., The balance sheet of metabolites for potato discs showing the effect of salts and dissolved oxygen on metabolism at 23° C. Plant Physiol. 15:409-448. 1940.

GAMETOPHYTES OF MARATTIA SAMBUCINA AND MACROGLOSSUM SMITHII

ALMA G. STOKEY

(WITH THIRTY FIGURES)

Introduction

The sporophytes of the Marattiaceae show considerable range in form and size, but the gametophytes are decidedly uniform. They are sufficiently different from the gametophytes of the leptosporangiate ferns as to be easily recognized. Descriptions varying in their fullness have been given of the gametophytes of the following species: *Angiopteris evecta* by LUERSSEN (13), FARMER (8), SCHMELZEISEN (14), and HAUPT (9); *A. pruinosa* and *Angiopteris* sp. by JONKMAN (11, 12); *Macroglossum alidae* by CAMPBELL (7); *Marattia cicutaefolia* by LUERSSEN (13); *M. fraxinea*, *M. weinmannifolia*, *M. kaulfussii*, and *M. verschaaffeltiana* by JONKMAN (11, 12); *M. douglasii* by CAMPBELL (3); *M. alata* by SCHMELZEISEN (14); *Danaea simplicifolia* by BREBNER (2); *D. jenmanii*, *D. elliptica*, and *D. jamaicensis* by CAMPBELL (5, 6); and *Kaulfussia aesculifolia* by CAMPBELL (4).

Material and methods

The spores of *Marattia sambucina* Bl. were collected by the writer from plants growing wild in a rich moist ravine in the forest near the Botanical Garden at Tjibodas, Java, in June, 1931, and July, 1937. The set of cultures started in 1931 grew for 2 years; the second set, started in Java in 1937, are still growing at Mount Holyoke College. The work on *Macroglossum smithii* (Rac.) Campbell is based on a set of cultures from spores collected in 1931 from a plant in the Botanical Gardens at Buitenzorg, which is doubtless the plant to which CAMPBELL refers in his discussion of the relation of *M. smithii* to *M. alidae* (7). Germination stages were obtained on distilled water and later stages on peat by methods described previously (15). Fresh prothalli were used for the study of germination stages, habit, rhizoids, external aspect, and distribution of sex organs. A weak chromo-acetic acid with a few drops of osmic acid gave the most satisfactory results in killing and fixing, but formalin-acetic acid and a modified Navashin's fluid were also good. It is more difficult to get satisfactory preparations of the prothalli of the Marattiaceae than of other families of ferns, probably because of the heavily cutinized cells surrounding the delicate internal tissues.

The early work was carried out in the Treub Laboratory, Buitenzorg, Java.

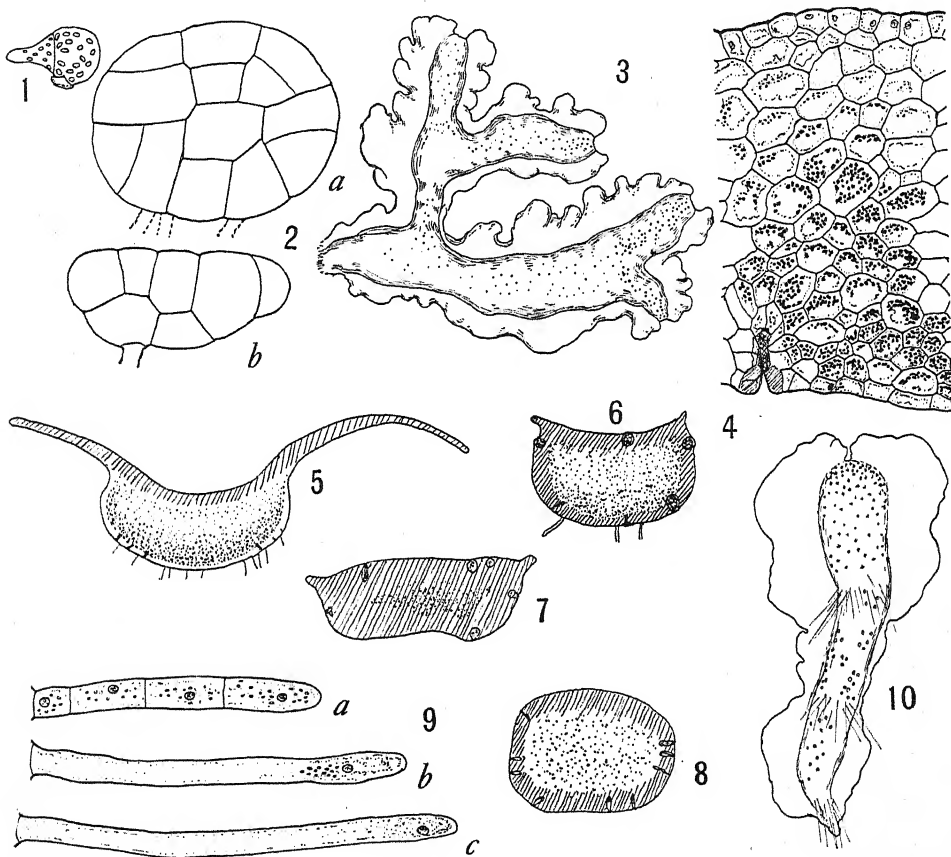
The cultures were brought to the United States and the work continued in the laboratories of Mount Holyoke College and the Marine Biological Laboratory, Woods Hole, Massachusetts.

Observations

SPORE GERMINATION AND VEGETATIVE STAGES

The spores of *Marattia sambucina* are pale yellowish tan, bilateral and elongated, with a lengthwise ridge. The wall is slightly thickened and roughened. No radiate spores were found, but there were many spores—neither elongated nor thickened—which appeared to be immature and which failed to germinate. The spores become distinctly green before the spore coat ruptures. The cracking of the coat was seen 16 days after planting. There was good germination and sufficient growth 25 days after planting to give the peat the characteristic dark green color of the Marattiaceae. There is great enlargement of the primary cell before the first division occurs; this usually cuts off the first rhizoid-bearing cell, although four or five cells may form before a rhizoid develops. The chloroplasts are conspicuous in the rhizoid and may persist for considerable time, not only in the primary rhizoid but in those formed much later (figs. 1, 9a, b). The next divisions result in a plate, and the plate develops into a mass. The division in the third plane may begin when there are only four cells, or it may not occur until later. The early thickening may not involve all the cells, as in figure 2, in which only the basal portion of the thallus is two cells thick. No case was seen in which there was a filamentous stage at germination, although there were many irregularities. The apical cell may appear just after the quadrant stage but usually not until later. The next stages lead gradually to the development of a heart-shaped thallus. A midrib is formed which is not necessarily continuous with the thickened base. The wings are two cells thick, except for the margin which is one cell thick and the region near the midrib which may be heavier. Like all the gametophytes of the Marattiaceae which have been described, they are notable for their dark green color. Crowded prothalli may be distinctly slender and have a blunt tip. No filamentous ameristic prothalli were found. When growth is continued for several years under varying conditions, there may be periods in which rib development is dominant and little or no wing is formed (figs. 6, 7, 8); but under conditions which are probably more favorable there is development of wing as well as midrib (fig. 5). The wings are usually flat, and while they may become irregular they are not ruffled as in *Gleichenia*. Branching is not uncommon in gametophytes several years old; and old gametophytes, branched or unbranched, usually show irregular margins. The branching prothallus in figure 3 was 27 months old and had four apices, three of which were producing archegonia. If fertilization does not occur, the gametophytes under culture conditions may grow indefinitely and in 3 or 4 years may attain a length of 25–35

mm. The structure of a midrib in a region in which there were many old archegonia is shown in figure 4. As is often the case in old prothallia, there is abundant storage of starch. The difference in size which commonly exists between the superficial and the internal cells may be noted. The layer of heavily cutinized cells on both dorsal



FIGS. 1-10.—Figs. 1-9, *Marattia sambucina*: Fig. 1, stage shortly after germination of spore. Fig. 2, two views of young prothallus, posterior portion two cells thick. Fig. 3, prothallus 27 months old. Fig. 4, cross section of midrib containing starch. Figs. 5-8, cross sections of prothalli through portions showing variation in midrib and wing development; shading indicates chlorophyll-bearing cells; stippling indicates starch. Fig. 9, young rhizoids from prothalli 4 years old; *a* and *b* contain disintegrating chloroplasts. Fig. 10, *MacroGLOSSUM smithii*, prothallus 13 months old bearing many archegonia and antheridia.

and ventral surfaces seems to constitute a physiological epidermis. Figure 5 shows a section cut from a fresh prothallus in which the wings and upper parts of the midrib were deep green, but the rest of the midrib was colorless and contained much starch, particularly in the lower layers of cells. The midrib may be green through-

out (fig. 7), only the surface layers may be green (figs. 6, 8), or only the upper layer (fig. 5). Figure 7 shows a thallus in which the starch was stored in the center, in cells smaller than those of the surrounding tissue, which were formed by secondary division of the large internal cells.

The rhizoids of *M. sambucina* are colorless, long, stout, and cutinized. In general they are limited to the midrib, and the production may be intermittent. A few old gametophytes were found to have septate rhizoids which were truly multicellular, with a nucleus in each cell (fig. 9a). The multicellular rhizoids consist of two to five cells and were found in the tufts of unicellular rhizoids, but were always in a minority. The production of septate rhizoids was not of rare occurrence in the old gametophytes examined but was not common enough to be considered characteristic. Young rhizoids—both unicellular and multicellular—containing chloroplasts were found on prothalli 3-4 years old (fig. 9a, b).

The presence of an endophytic fungus has been reported for the gametophytes of the Marattiaceae collected in the open. Naturally no fungus was found in the gametophytes under investigation, which were grown on sterilized peat. Their vigorous growth and normal development support the view that the fungus is of little or no importance in the life of the gametophyte.

Regeneration occurs vigorously under certain conditions. The margin or even the surface of an old thallus may develop such a large number of regenerated growths as to suggest a rosette. The buds usually form a quadrant, much like a young thallus growing from a spore, and then—if space permits—the development proceeds as in a young prothallus (figs. 26, 27). Crowding prevents symmetrical development in many cases. Thickening of the posterior portion of the new thallus may occur early, with the anterior portion remaining one cell thick for a considerable time and sometimes broadening into a fanlike form by marginal growth. The regenerated branch may give rise to other regenerated branches before separation from the parent thallus takes place. In general, the rhizoids develop later than on similar structures in the Polypodiaceae. Small antheridia may appear on the young regenerated growths before separation from the parent thallus is complete (fig. 30).

The spores of *Macroglossum smithii* are of the radiate type and are pale in color. No elongated spores were seen. Germination was abundant. The germination and early stages were followed less closely than those of *Marattia sambucina*, but all stages seen suggest the same range in form and the same type of development from germination of the spore until maturity of the sex organs. The gametophytes have the deep green color characteristic of the family. The wings, while not perfectly regular, are not curled or crisped but usually lie flat. The midrib may become very heavy, and the proportion of wing to midrib varies under certain undetermined conditions, as in *M. sambucina* (fig. 10). Unlike *Marattia*, the wings tended to

remain one cell thick, as CAMPBELL noted for *Macroglossum alidae*; but no prothallia were in culture as long as those of *Marattia*. The rhizoids are long and colorless, holding the thallus close to the ground. No multicellular rhizoids were found.

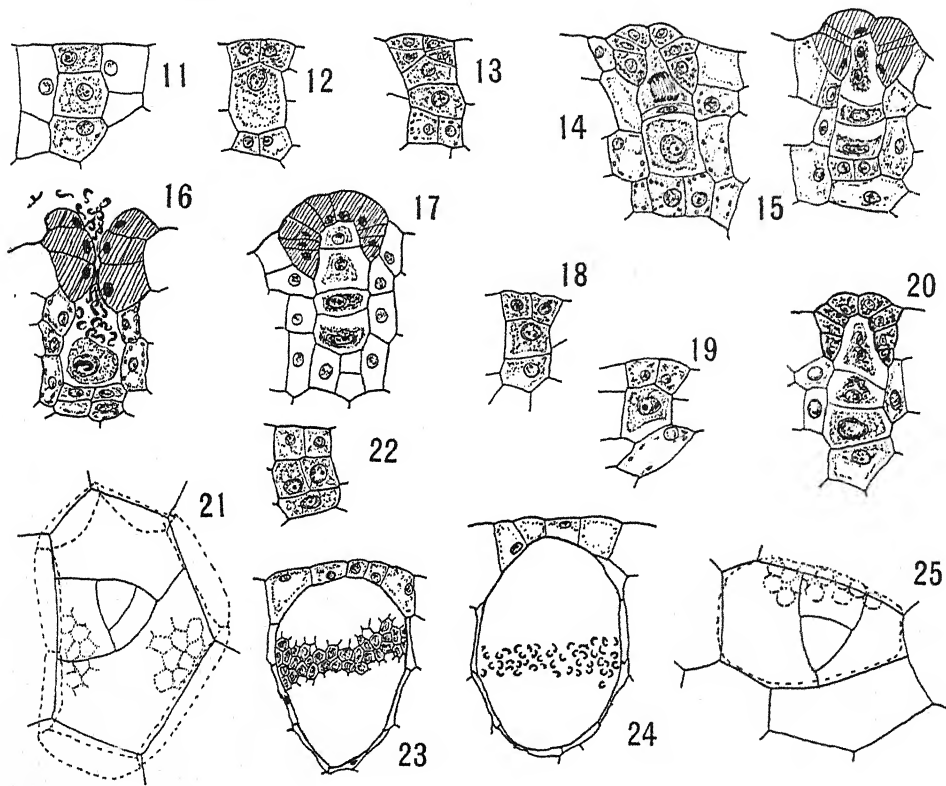
REPRODUCTIVE STRUCTURES

Antheridia were abundant on prothalli of *Macroglossum* 5 months old but did not appear on *Marattia* until the prothalli were 8 months old, and they were never quite as abundant as on *Macroglossum*. They appear first on the ventral side but may soon be found on the dorsal side as well. Archegonia were found on both species when the gametophytes were about 1 year old. As all the cultures were started in June or July and brought to the United States in September, it is possible that the weaker light of late autumn and early winter retarded the development of the prothalli and delayed the formation of sex organs. The production of antheridia usually continues throughout the life of the gametophyte, but after the production of archegonia begins the antheridia may develop chiefly on the dorsal surface. Antheridia may continue to form when conditions become unfavorable for the formation of archegonia.

The development of the antheridium of the two species is apparently just like that described for other species of the Marattiaceae. It is initiated in the primary antheridial cell by a periclinal division, which separates the primary cover cell from the primary spermatogenous cell (fig. 22). In the formation of small or medium-sized antheridia the primary cover cell undergoes three divisions, and four in the case of large antheridia, forming in the center a triangular opercular cell (figs. 21, 25). Secondary divisions may occur in one or more of the cover cells surrounding the opercular cell. There is a great range in the size of mature antheridia. Antheridia on regenerated branches may show only 15 to 30 sperms in a median section, but on vigorous young gametophytes the number is likely to range from 50 to 80, while on vigorous large prothalli the range may be from 140 to 170, a number considerably larger than that usually credited to the Marattiaceae. The antheridia shown in figures 23 and 24 are not exceptional for *Macroglossum smithii*, and antheridia equally large or larger may be found on *Marattia sambucina*. The spermatocyte mass is surrounded by a mantle or jacket layer cut off from the surrounding cells—except on the side adjoining the cover cells (figs. 21, 23, 24). In longitudinal section it can be seen that the mantle layer is sometimes incomplete.

Stages in the development of the archegonium of *Marattia sambucina* are given in figures 11–16. In the development of the archegonium of *M. sambucina* a basal cell was found to be a characteristic feature. In *Macroglossum smithii*, on the contrary, the archegonium more frequently develops without a basal cell (fig. 19), although such a cell may form occasionally (fig. 18). The neck and axial row are

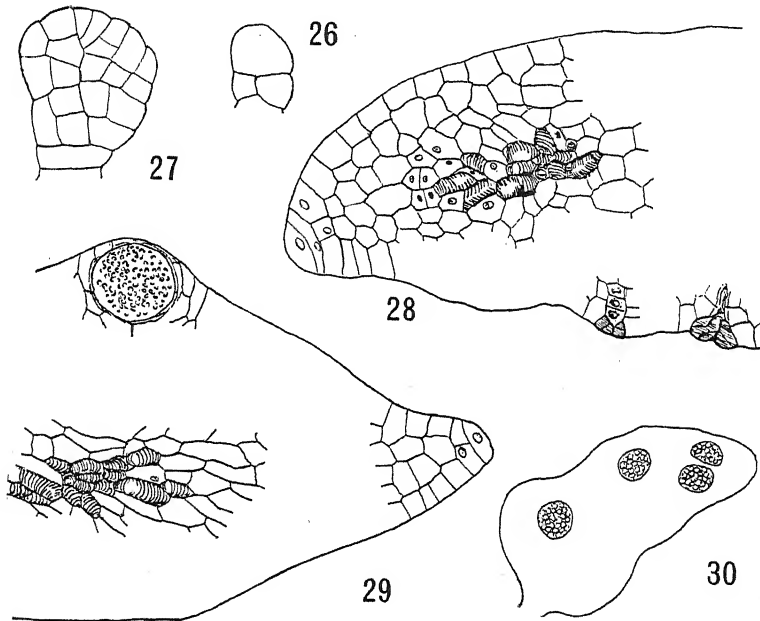
formed by the usual divisions of the primary cells, as given in detail by HAUPT (9). The neck of both species, as in other members of the family, is very short and scarcely projects above the surface. The flatness is a result of the form and size of the cells rather than of their number. The neck consists of four rows of cells, and



FIGS. 11-25.—Figs. 11-17, *Marattia sambucina*: Figs. 11-16, series in development of archegonium. Fig. 17, archegonium with two neck canal cells. Figs. 18-24, *Macroglossum smithii*: Fig. 18, early stage of archegonium with basal cell. Fig. 19, without basal cell. Fig. 20, mature archegonium. Fig. 21, surface view of antheridium with four divisions in primary cover cell; jacket layer indicated by dotted lines. Fig. 22, early stage of antheridium. Figs. 23, 24, longisection of antheridia from gametophytes 16 months old. Fig. 25, *Marattia sambucina*, surface view of antheridium with three divisions in primary cover cell.

each row consists usually of three cells; but the middle cell frequently divides, forming two cells which are apt to be flatter than the outer and inner cells. Occasionally there are five cells in a row (fig. 17). The number of cells is not always the same in all rows, but no consistent pattern in the variation was found. The walls of the neck cells become modified as the archegonium matures, and the cell contents undergo changes such that stained preparations of even the early stages may show coarse granules, and those of the later stages may show little or no differentia-

tion except for the nucleus. The axial row is short and more uniform in diameter than is usual in ferns. The egg is relatively small and the ventral canal cell relatively large, almost the size of the egg (figs. 15, 17, 20). A thin ventral canal cell is unusual (fig. 14). The mature archegonium usually shows two nuclei in the neck canal; but in several gametophytes of *Marattia*, archegonia were found in which there was a definite wall in the neck canal, forming two neck canal cells (fig. 17). In some cases the presence of two distinct protoplasts—instead of the customary



FIGS. 26-30.—*Marattia sambucina*: Figs. 26, 27, young regenerated branches. Figs. 28, 29, apogamy Fig. 30, regenerated branch bearing small antheridia.

single mass in the neck—indicated that a delicate wall had been present but had disappeared or was not revealed in the preparation. Fewer preparations were made of *Macroglossum*, and only one case was found with two neck canal cells. The jacket layer of nutritive cells around the archegonium venter is a conspicuous feature in leptosporangiate ferns but is less prominent in the Marattiaceae. In both species under investigation the jacket layer was late in development and never was as prominent as in other ferns. The basal cell, when present, divides early, and the resulting cells form the most conspicuous part of the jacket layer. Since in *M. smithii* the basal cell is usually lacking, the jacket layer is more belated than in *Marattia sambucina* and is even less conspicuous. At the time of fertilization there is usually a complete layer, but it may consist of so few cells that not

more than six will be seen in longitudinal section. The contents of the jacket cells are less dense than in leptosporangiate ferns, and the cells are more or less tabular in form rather than cubical (figs. 15-17). In both *Marattia* and *Macroglossum*, archegonia were found into which large numbers of sperms had penetrated (fig. 16). As might be expected, the robust gametophytes of these ferns can support two sporophytes for considerably longer than the more delicate gametophytes of the leptosporangiates. In the case of branched prothalli, the branches act as independent units, and even on unbranched prothalli two sporophytes may continue to grow for considerably longer than would be the case in other families of ferns.

APOGAMY

Two cases of apogamy were found in *Marattia sambucina*, both consisting in the development of tracheids in the thallus. A gametophyte 27 months old had produced both antheridia and archegonia of normal appearance (fig. 28). In this thallus there were two distinct masses of tracheids found in different sequences of sections. The gametophyte (fig. 28) was more than 3 years old and had produced many apparently normal antheridia and archegonia. These gametophytes, like all the others, had been grown on peat with a moderate degree of uniformity of moisture, in a glass culture dish with a high degree of humidity, and were exposed to the light in a north window.

Discussion

The following may be considered distinguishing features of the gametophytes of the Marattiaceae: germination of the spore into a plate and then into a mass; slow development and relatively large size and thickness of the thallus; deep green color; rhizoids which are colorless in most species and which may be septate; imbedded antheridium with a large output of sperms; a short broad archegonium with a neck which scarcely projects and with a poorly developed jacket layer.

The filamentous stage at germination of the spore, which is so common in higher ferns, is extremely rare but has been reported by JONKMAN (11, 12). The failure to develop a filament under adverse conditions, particularly of light (which induces it in most ferns), indicates a lack of plasticity which may be a decided handicap. The deep green color of the gametophyte is sufficiently uniform as to be distinctive of the family. The shade of green, combined with the large size and unusual thickness of the thallus, gives the gametophyte the appearance of a liverwort, with the colorless rhizoids contributing to the resemblance. JONKMAN (11, 12), who worked on four species of *Marattia* and two of *Angiopteris*, states that the rhizoids are colorless and do not become brown, even on very old prothalli. CAMPBELL (4) describes the rhizoids of *Kaulfussia* as being stout and thick with colorless walls, but he states that the rhizoids of *Danaea* and also of *Marattia* and *Angiopteris* are

brown in color (6). SCHMELZEISEN (14) speaks of the many chloroplasts in the rhizoids of *Angiopteris evecta*. He states that the rhizoids arise by the differentiation of ordinary prothallial cells, and that there are all degrees of differentiation between the rhizoid and the assimilating cell. Colorless rhizoids occur in many if not in most species of the Marattiaceae but are exceptional in other families of ferns. In the leptosporangiate ferns, as a rule, the rhizoids are colorless only in the young stage or on very young gametophytes, while on the mature gametophytes the range in color is from the pale tan of many species of the Polypodiaceae to a distinct brown such as is found in the Gleicheniaceae and the Hymenophyllaceae. The presence of conspicuous chloroplasts in the young rhizoids of young gametophytes of ferns with chlorophyll-bearing spores, such as those of *Osmunda*, has often been noted; but they may also occur in species without chlorophyll in the spore, as in many species of *Gleichenia*. In the higher ferns, except in the species with green spores, the chloroplast-bearing stage is very short if it occurs at all. The multicellular rhizoids which occur occasionally in *Marattia sambucina* are characteristic of the four species of *Danaea* which have been investigated (2, 5, 6). The high development of cutin which is characteristic of the superficial cells of the thallus is also characteristic of the rhizoids of the Marattiaceae. In general, the characteristics of the rhizoids of the Marattiaceae—prevalence of colorless rhizoids, persistence of chloroplasts, tendency to septation, and heavy cutinization—indicate that they are less highly differentiated from the other cells of the thallus and are less highly specialized than are the rhizoids of the leptosporangiate ferns.

The tendency for the thallus under certain undetermined conditions to develop as a subterete structure may throw some light on the origin of the Ophioglossaceae. The storage of starch is probably related to the relatively long life of the gametophyte of the Marattiaceae in contrast to that of higher ferns.

The archegonium is peculiar in the deeply set neck with only a slight projection, since in both the Ophioglossaceae and the leptosporangiate ferns the neck projects distinctly, even when—as in the Hymenophyllaceae—there are only four cells in a row. In regard to the ventral canal cell the family shows the two extremes, from the very small short-lived cell in *Danaea* to the condition in the other genera in which the ventral canal cell is much larger in proportion to the egg than in the leptosporangiate ferns. A wall between the neck canal nuclei has been reported for the Marattiaceae more frequently than for any other family. Within the family there is variation in the presence or absence of a basal cell, but the character does not appear to be consistent, even in a single species. The relatively slow and weak development of the jacket layer, noted first in the work of JONKMAN (11, 12), may be a handicap if it affects the early development of the embryo.

Apogamy has been reported for four families of the leptosporangiate ferns, but the only case known for the eusporangiates is in *Botrychium virginianum* described

by JEFFREY (10). In *Botrychium*, as in *Marattia*, it consisted in the formation of tracheids in the gametophyte. BEYERLE (1) reported apospory in *M. alata* consisting in the production experimentally of rhizoids on primary leaves.

Summary

1. Germination of the spores of *Marattia sambucina* and *Macroglossum smithii* results first in a plate and then in a mass. The gametophytes are dark green from the very first, but the old gametophytes may show a pale tissue in the midrib—which may contain a large amount of starch. The rhizoids of both species are colorless; multicellular rhizoids are produced occasionally by *Marattia sambucina*.

2. The antheridia show wide range in size, from those on regenerated branches which may show 15-30 sperms in median section to those on vigorous gametophytes 1-4 years old which may show 140-170 sperms.

3. The archegonium of both species is short and broad, projecting but slightly beyond the surface. The two nuclei of the neck canal are usually not separated by a wall, but occasionally a wall is formed. The ventral canal cell is large. The jacket layer consists of a few tabular cells which are slow in developing and not highly differentiated before fertilization.

4. Apogamy, shown in the development of tracheids, was found in two gametophytes of *Marattia sambucina* which had borne both antheridia and archegonia.

The writer wishes to express her thanks to the members of the staff of the Botanical Gardens at Buitenzorg and Tjibodas, Java, for their generous assistance in facilitating the collection and culture of the material used in this investigation.

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LITERATURE CITED

1. BEYERLE, R., Untersuchungen über die Regeneration von Farnprimärblättern. *Planta* 16: 622-665. 1932.
2. BREBNER, G., On the prothallus and embryo of *Danaea simplicifolia* Rudge. *Ann. Bot.* 10:109-122. 1896.
3. CAMPBELL, D. H., Observations on the development of *Marattia Douglasii* Baker. *Ann. Bot.* 8:1-20. 1894.
4. ———, The prothallia of *Kaulfussia* and *Gleichenia*. *Ann. Jard. Bot. Buitenzorg.* 2d ser. 8:69-102. 1908.
5. ———, The prothallia and embryo of *Danaea*. *Ann. Bot.* 23:691. 1909.
6. ———, The Eusporangiatæ. *Carnegie Inst. Wash. Publ. no. 140.* 1911.
7. ———, The structure and affinities of *Macroglossum Alidae* Copeland. *Ann. Bot.* 28:651-669. 1914.
8. FARMER, J. B., On the embryogeny of *Angiopteris evecta*. *Ann. Bot.* 6:265-270. 1892.

9. HAUPT, A. W., Sex organs of *Angiopteris evecta*. Bull. Torr. Bot. Club 67:125-129. 1940.
10. JEFFREY, E. C., The gametophyte of *Botrychium virginianum*. Univ. Toronto Biol. Studies 1:1-32. 1898.
11. JONKMAN, H. F., Ueber die Entwicklungsgeschichte des Prothalliums des Marattiaceen. Bot. Zeitung 36:129-136; 145-153. 1878.
12. ———, La génération sexuée des Marattiacées. Arch. Néerland 15:198-224. 1880.
13. LUERSSEN, C., Ueber die Entwicklungsgeschichte des Marattiaceen Vorkeims. Bot. Zeitung 33:535-547. 1875.
14. SCHMELZEISEN, WILHELM, Beiträge zur Entwicklungsgeschichte der Prothallien einiger Marattiaceen, Cyatheaceen, und Polypodiaceen. Flora 27:46-80. 1933.
15. STOKEY, ALMA G., Prothallia of the Cyatheaceae. BOT. GAZ. 90:1-45. 1930.

AUXINS IN SOME AMERICAN SOILS

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Since auxins have been found in plant tissues it is reasonable to suppose that they may persist through decomposition stages of organic matter and become a part of the soil, or perhaps they may be synthesized during the decomposition process. The report presented here deals with the extraction of auxin from some virgin American soils and its relation to other soil characteristics. What this means in relation to plant growth is left for future consideration.

In this investigation the standard *Avena* test was used to measure the auxin extracted from the soils. The results are expressed as indoleacetic acid equivalents. This is an arbitrary term and does not imply that indoleacetic acid was the auxin extracted from the soil.

Various methods have been used for auxin extraction from different kinds of substances, but freshly purified peroxide-free ethyl ether has been a popular solvent. This background of experience was utilized in the development of a method adapted to extraction of soil auxins. The surface horizons of Cecil, Russell, and Barnes soils were used for a considerable number of preliminary experiments to find a satisfactory way of extracting comparable quantities of auxin. A résumé of these various tests follows:

1. Ether or alcohol used alone extracted little or no auxin, at least from acid soils.
2. Acidifying a soil-water mixture to a pH of 4.5 with hydrochloric or acetic acid, with or without refluxing, on an electric hotplate gave no auxin in the water-ether extract.
3. Mixtures of ether and water extracted more auxin from acid soils than either solvent alone, but approximately the same amount was extracted by water as by ether-water mixtures when the pH was slightly alkaline.
4. Water extraction at pH 7-8 formed the basis of the method adopted. This method is as follows.

One hundred gm. of air-dry soil in an 800-cc. beaker was treated with 200 cc. of water and enough limewater added to produce a final pH of about 7.5. About 30 per cent more limewater was needed than that required to hold such a pH for the first 2 hours, because of the slow reaction with soil acids. Water was then added to make the total volume of liquid 400 cc. After standing with occasional

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stirring for 48 hours, the total extraction period, the suspension was filtered on a Büchner funnel through no. 1 Whatman filter paper, and the filtrate evaporated to dryness in a casserole over a steambath. Twelve cc. of water was then added and the walls carefully policed down. This solution was transferred to a 250-cc. wide-mouthed Erlenmeyer flask and 120 cc. of ether added; the flask was stoppered, shaken at intervals, and stored in an icebox over night.

TABLE 1
RELATION OF AUXIN TO OTHER PROPERTIES IN VIRGIN SOILS

NAME AND LOCATION OF SOIL*	LABORATORY NO.	HORIZON	DEPTH (INCHES)	pH	ORGANIC MATTER CONTENT† (%)	INDOLEACETIC EQUIVALENTS IN MICROGRAMS PER KG. OF SOIL	
						SAMPLE 1	SAMPLE 2
Barnes loam, South Dakota	C2929.....	A	0-9	6.9	5.98	0.165	0.134
	C2930.....	B ₁	9-17	7.1	2.36	0.161	0.169
Brookston silty clay loam, Indiana	C4043.....	A ₁	0-6	6.8	10.42	0.112	0.113
	C4044.....	A ₂	6-20	6.4	2.67	0.148	0.196
Carrington loam, Iowa	C2916.....	A ₁	0-3	5.5	5.57	0.106	0.141
	C2917.....	A ₂	3-13	5.2	3.43	0.122	0.154
Miami silt loam, Indiana	C4060.....	A ₁	0-2	6.3	6.22	0.084
	C4061.....	A ₂	2-5	5.9	3.60	0.034
Russell silt loam, Indiana	C3174.....	A ₁	0-2	5.8	6.26	0.125	0.118
	C3175.....	A ₂	2-8	4.5	2.94	0.000	0.039
Hagerstown silty clay loam, Pennsylvania	C 798.....	A ₁	0-2	4.5	11.04	0.043	0.068
	C 799.....	A ₂	2-8	4.8	2.53	0.000	0.026
Hillsdale fine sandy loam, Michigan	C4031.....	A ₁	0-3	7.5	4.28	0.118	0.072
	C4032.....	A ₂	3-9	7.5	0.72	0.000
Decatur silty clay loam, Georgia	C7178.....	A	0-12	6.3	4.05	0.132	0.100
	C7179.....	B ₁	12-36	5.7	0.46	0.000	0.026
Cecil fine sandy loam, South Carolina	C7173.....	A ₁	0-2	6.1	4.16	0.069	0.099
	C7174.....	A ₂	2-8	5.1	0.85	0.000	0.067
Georgeville silt loam, North Carolina	C7186.....	A ₂	$\frac{1}{2}$ -5	4.5	3.44	0.097	0.092
	C7187.....	B ₁	8-28	4.7	0.23	0.000	0.000
Norfolk fine sandy loam, North Carolina	C7190.....	A ₁	0-2	4.4	10.52	0.038
	C7191.....	A ₂	2-13	5.3	0.43	0.000	0.000

* Soils arranged in order of approximate native fertility.

† Determined by combustion, CO₂X0.471.

The following day the ether was carefully decanted from the solution into another Erlenmeyer flask of similar size and evaporated on a water-bath to 3-5 cc. This volume of ether was then further evaporated drop-wise by means of a pipette on 0.3 cc. of 1.5 per cent agar contained in a 5-cc. shell vial. During evaporation, the vial was suspended in a rapidly boiling water-bath. The flask was carefully rinsed twice with 2-cc. portions of fresh ether and these likewise evaporated on the agar. Then the walls of the shell vial were carefully washed down with fresh ether. By this procedure auxin from a 48-hour water extract of soil was concentrated in 0.3 cc. of 1.5 per cent agar. This volume of agar was molded into

twelve blocks, suitable for auxin determination by the *Avena* test. Inasmuch as the amounts of auxin obtained during this procedure caused *Avena* curvatures that were less than the maximum angle (30°), auxin determinations at dilutions of the original extract were not made.

The general characteristics of most of the soils used have been summarized (19). The soils are typical specimens of eleven soil series representing four of the great soil groups. Each soil was air-dried and passed through a 2-mm. sieve. The soils had been stored for periods varying from 3 months to 4 years. Data for these soils are given in table 1. In table 2 more detailed data obtained in the *Avena* test are given. While the soils are listed in the order of their approximate fertility, this listing should be considered as having only general significance. These tables, together with the preliminary results, not given in detail, show that the extractable auxin content tends to decrease with increasing soil acidity and that surface and second horizons of soils of excellent native fertility tend to have higher extractable auxin contents than do the second horizons of less fertile soils.

It is recognized that determination of the auxin content of soils by the procedure described does not possess the same degree of precision as is involved in the determination of most of the inorganic chemical constituents. It is not known what part of the auxin present is extracted by the means used, nor is it known to what extent—if any—auxin is created or destroyed by the extraction process which is carried out under nonsterile conditions. The important fact is that fairly concordant duplicate determinations were obtained when extractions were made at different times and evaluated with different sets of oat plants separately standardized as to sensitivity to auxin.

These data are in accord with those obtained by PARKER-RHODES (12), whose study of cultivated soils constitutes the only previous work on determination of soil auxin. He used a root-hair plasmolysis technique to estimate the amounts of auxin. In terms of indoleacetic acid, concentrations of 10^{-9} to 10^{-10} moles per kilogram of soil are reported. He suggests the auxin from soil has certain characteristics of indoleacetic acid, but at present the chemical compound or compounds acting as soil auxin(s) must be regarded as unknown.

From auxin determinations of soil microorganisms, ROBERTS and ROBERTS (13) conclude that the soil flora is potentially capable of producing appreciable amounts of auxin. The work of KÖGL and KOSTERMANS (6), THIMANN (16), and others indicates that the auxin produced by many of these microorganisms is indoleacetic acid.

It has been reported that 10^{-9} molar indoleacetic acid stimulates root growth of several kinds of plants (1, 2, 17). Since this optimum concentration of auxin for root growth is found only in the fertile soils, it may be one of the factors in some way contributing to their fertility. Furthermore, the preliminary experi-

TABLE 2

CURVATURE OF AVENA COLEOPTILES IN RELATION TO AUXIN EXTRACTED FROM VIRGIN SOILS
(AS DEGREES, AND AS MICROGRAMS OF INDOLEACETIC ACID EQUIVALENTS
PER KG. OF SOIL, BRACKETED NUMBERS)

SOIL TESTED	HORI- ZON	DEPTH (INCHES)	DATE OF AVENA TEST							
			10/4	10/6	10/7	10/9	10/10	10/11	10/16	10/17
Barnes, South Dakota	A	0-9	22.6 (0.134)	17.6 (0.165)
	B ₁	9-17	18.5 (0.109)	18.0 (0.169)	20.1 (0.161)
Brookston, Indiana	A ₁	0-6	17.0 (0.112)	11.3 (0.113)	18.3 (0.147)
	A ₂	6-20	24.4 (0.196)	14.8 (0.148)	25.2 (0.202)
Carrington, Iowa	A ₁	0-3	17.9 (0.106)	17.6 (0.141)
	A ₂	3-13	20.5 (0.122)	24.7 (0.172)	22.0 (0.154)
Miami, Indiana	A ₁	0-2	12.1 (0.084)
	A ₂	2-5	4.9 (0.034)
Russell, Indiana	A ₁	0-2	20.0 (0.118)	17.9 (0.125)
	A ₂	2-8	0.00 (0.000)	4.2 (0.039)
Hagerstown, Penn- sylvania	A ₁	0-2	6.5 (0.043)	9.6 (0.068)
	A ₂	2-8	0.00 (0.000)	3.7 (0.026)
Hillsdale, Michigan	A ₁	0-3	8.8 (0.072)	11.8 (0.118)
	A ₂	3-9	0.0 (0.000)
Decatur, Georgia	A	0-12	16.2 (0.132)	14.0 (0.100)
	B ₁	12-36	0.00 (0.000)	3.6 (0.026)
Cecil, South Caro- lina	A ₁	0-2	8.4 (0.069)	14.2 (0.099)
	A ₂	2-8	0.0 (0.000)	6.7 (0.067)	12.3 (0.086)
Georgeville, North Carolina	A ₂	½-5	0.0 (0.000)	9.8 (0.092)	12.2 (0.097)
	B ₁	8-28	0.0 (0.000)	0.0 (0.000)
Norfolk, North Carolina	A ₁	0-2	5.7 (0.038)
	A ₂	2-13	0.0 (0.000)	0.0 (0.000)	0.0 (0.000)
Indoleacetic acid, 50 micrograms/li- ter.....	22.5	18.4	25.4	15.0	21.1	16.0	18.7	21.5

ments mentioned indicate that the pH of these soils should not be destructive to auxin, as a more acid soil would be expected to be.

Recently LAUDE (8) has shown that a 10^{-9} molar solution of indoleacetic acid, when applied to tomato plants grown in washed quartz sand and with added inorganic nutrients, increased their dry weight 12.5 per cent more than control plants receiving the inorganic nutrients alone. Again the concentration of auxin in the fertile soils seems to approach this optimum.

While there are several claims that indoleacetic acid and other auxins added to soil stimulate growth (3, 18, 9, 14, 5, 10, 20), there are nearly as many claims that this is not so (11, 15, 7, 4). The present data make no case for the desirability of adding auxin to soil as a means of stimulating plant growth, but they indicate that fertile soils tend to contain more auxin than less fertile ones, and—except in the most fertile—surface soils more than the subsoils. This suggests that natural soil auxin may, under certain conditions, have some stimulative effect upon plant growth. Also it offers the possibility of an additional partial explanation for the infertility of subsoils so frequently noted.

Summary

1. A proximate method is described for the extraction and assay of auxin in soils. One hundred gm. of soil is shaken at intervals for 48 hours with sufficient limewater to keep the pH between 7 and 8 in a volume of 400 cc. The filtered extract is then concentrated and auxin measured by the standard *Avena* test.

2. Auxin determinations were made on the two upper horizons of eleven virgin soils representative of four of the great soil groups. In very fertile midwestern surface soils and subsoils auxin was found to the extent of about 0.175 micrograms per kilogram of soil (indoleacetic acid equivalents). In less fertile soils considerably less auxin was found in the surface horizons and little if any in the second horizon.

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LITERATURE CITED

1. AMLONG, H. U., Der Einfluss des Wuchsstoffes auf die Wanddehnbarkeit der *Vicia faba* Wurzel. Ber. Deutsch Bot. Ges. 54:271-275. 1936.
2. FIEDLER, H., Entwicklungs- und reiz physiologische Untersuchungen an Kulturen isolierten Wurzelspitzen. Zeitschr. Bot. 30:385-436. 1936.
3. GRACE, N. H., Physiological curve of response to phytohormones by seeds, growing plants, cuttings, and lower plant forms. Canad. Jour. Res. 15:538-546. 1937.
4. HABER, C. S., and EDGECOMBE, S. W., Influence of vitamin B₁ and other growth promoting substances on growth of plants. Ann. Rep. (Trans.) Iowa State Hort. Soc. 75(1940):142-153. 1940.

5. JONES, F. D., Hormone treatment of grass seed. National Seedsman. October, 1940.
6. KÖGL, F., and KOSTERMANS, D. G. F. R., Hetero-auxin als Stoffwechselprodukt niederer pflanzlicher Organismen. Isolierung aus Hefe. XIII Mitteilung. Zeit. Physiol. Chem. 228: 113-121. 1934.
7. LAFFERTY, H. A., The effect of certain hormones on barley. Proc. Intern. Seed Test. Assoc. 12:19-21. 1940.
8. LAUDE, H. M., Combined effects of potassium supply and growth substances on plant growth. BOT. GAZ. 103:155-167. 1941.
9. McEVOY, E. T., The response of tobacco seedlings to the growth promoting substance, "heteroauxin." The Lighter 9:14-16. 1939.
10. MELL, C. W., Treating grass and clover with rootone. Seed World. October, 1941.
11. MITCHELL, J. W., Effect of indoleacetic acid on the growth of some crop plants. Proc. Amer. Soc. Hort. Sci. 36:171-176. 1939.
12. PARKER-RHODES, A. F., Preliminary experiments on the estimation of traces of heteroauxin in soils. Jour. Agr. Sci. 30:654-671. 1940.
13. ROBERTS, J. L., and ROBERTS, C., Auxin production by soil microorganisms. Soil Sci. 48: 135-140. 1939.
14. STIER, H. L., and DU BUY, H. G., The influence of certain phytohormone treatments on the time of flowering and fruit production of tomato plants under field conditions. Proc. Amer. Soc. Hort. Sci. 36:723-731. 1939.
15. TEMPLEMAN, W. G., and MARMOY, C. S., The effect upon the growth of plants of watering with solutions of plant growth substances and of seed dressings containing these materials. Ann. Appl. Biol. 27:453-471. 1940.
16. THIMANN, K. V., On the plant growth hormone produced by *Rhizopus suinus*. Jour. Biol. Chem. 109:279-291. 1935.
17. THIMANN, K. V., Auxins and the growth of roots. Proc. Nat. Acad. Sci. 22:511-514. 1936.
18. THIMANN, K. V., and LANE, R. H., After-effects of the treatment of seeds with auxin. Amer. Jour. Bot. 25:535-543. 1938.
19. UNITED STATES DEPARTMENT OF AGRICULTURE. Soils and Men. Yearbook. 1938.
20. WEIL, LIONEL, Fertilizer and fertilizer additive. U.S. Pat. no. 2,229,948. January 28, 1941.

EFFECT OF PHENYLACETIC ACID AND NAPHTHALENE ACETAMIDE ON TOMATO PLANTS GROWN IN SOIL¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 539

MERLE EDISON HAMNER

Introduction

There are many reports on the growth-promoting effects of some of the growth-regulating substances when applied to plants growing in soil. In relatively few of these do the results presented lend themselves to an actual statistical examination. Often no detailed data of any kind are given. Recently LAUDE (2) presented data which showed that, when grown in sand culture and under certain conditions of potash supply, there may be an increased growth of red kidney bean plants when very small quantities of indoleacetic acid are supplied in the nutrient medium. When under similar conditions small quantities of naphthalene acetamide were supplied instead of indoleacetic acid, the growth of the tops was significantly reduced, but the dry weight of the roots was increased. SWARTZ (4) also found that in sand culture, marigold and cosmos showed either a decrease or no significant increase when small amounts of vitamin B₁, nicotinic acid, naphthaleneacetic acid, or combinations of these substances were added to the nutrient solution. C. L. HAMNER (1), using sand culture and either complete nutrient solution or one low in phosphate content, also found increased root growth and decreased top growth of bean plants when naphthalene acetamide was supplied in the culture solution. There was some evidence of increase in top growth of tomato plants when phenylacetic acid was present in the culture solution in a concentration of 10^{-8} , but such evidence was not conclusive. The higher concentrations under similar conditions resulted in lesser growth. Thus it appears reasonably certain that when certain of the growth-regulating substances are supplied in relatively high dilution in the nutrient solution to sand cultures, there may be an increase in growth over the controls.

Recently STEWART and ANDERSON (3) have shown that it is possible to extract auxins from certain native soils in sufficient quantity to be detectable by the *Avena* test, and at a concentration such that they might possibly affect the quantity and rate of growth of plants grown in such soils. In view of the data just mentioned, and those in many other reports, it was decided to test the possible effects

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of phenylacetic acid and naphthalene acetamide on plants grown in a native garden soil and also in another soil to which sand and calcium carbonate were added.

Experimentation

Two soils were selected. Soil I was a fertile garden soil from Lake Geneva, Wisconsin. Abundant crops of flowers and vegetables had been grown on it for several successive years. It was high in thoroughly decayed native organic matter, since it came from low land near the lake shore. Upon testing, it was found to have a pH of 7.3. Soil II was basically a clay loam from near Chicago, and on it also crops of vegetables had been grown for several years. It was dark in color and fairly high in organic matter. After drying, this soil was crushed into fine pieces and then mixed with quartz sand on the basis of one part sand to five parts soil by volume. To this combination finely powdered calcium carbonate was added at the rate of 5 pounds of carbonate to each $1\frac{1}{2}$ cubic yards of soil. This combination was very thoroughly mixed. Upon testing it had a pH of 7.8.

Several hundred glazed crocks of $1\frac{1}{2}$ -liter capacity with side drainage were filled with these respective soils. They were arranged in rows of eight pots each. The pots containing one type of soil were distributed on two benches and those of the other type on two other benches, in a large greenhouse. The total number of crocks used was divided into twelve lots. Six lots contained soil I and six, soil II. Each lot consisted of ten rows of eight crocks. Five rows were used for the four concentrations of phenylacetic acid and its control and five for the naphthalene acetamide. The rows in each lot were randomized by successive drawings by chance of the numbers 1 to 5, to conform with the concentrations of growth-regulating substances used, and the rows were labeled accordingly, the rows of any specific concentration of the two substances standing side by side. Each pot was thoroughly watered with distilled water and allowed to stand several days before anything was planted in it.

On October 2, 1941, Bonny Best tomato seeds were planted in previously unused quartz sand and then lightly watered with distilled water. Thirteen days later, when the seedlings were about $1\frac{1}{2}$ inches high, the cotyledons spread apart but the epicotyl scarcely showing signs of elongation, uniform plants were selected and two were planted in each of the glazed crocks already filled with the respective soils. Later they were thinned to one plant per crock. The control plants grew well for the season of the year and were just coming into flower when harvested.

To avoid the use of tap water or distilled water in watering the plants, or as a medium in which to supply the growth substances, a complete nutrient solution—consisting of 0.0045 mol. MgSO_4 , 0.0045 mol. KH_2PO_4 , 0.0060 mol. $\text{Ca}(\text{NO}_3)_2$, together with the minor elements—was made up. This was then diluted to one part in twenty with distilled water. Whenever the plants were watered or the

growth substances were supplied, 100 cc. of the diluted medium with or without the growth substances added was used per crock.

The two growth substances tested were phenylacetic acid (Eastman) and naphthalene acetamide (American Chemical Paint Company). A concentrated solution was made up by dissolving the crystals in 95 per cent ethyl alcohol, and

TABLE 1
DRY WEIGHTS IN GRAMS OF THE SEVERAL RANDOM SAMPLES AT TIME OF HARVESTING

LOT	SUBSTANCE AND CONCENTRATION									
	0		10^{-10}		10^{-8}		10^{-6}		10^{-4}	
	CONTROL	CONTROL	N*	P*	N	P	N	P	N	P
SOIL I										
III.....	8.9	10.2	7.3	8.3	7.6	8.2	7.5	8.1	2.9†	6.7
IV.....	9.4	9.7	8.9	8.5	8.0	8.1	7.9	8.9	3.3†	6.8
V.....	8.9	9.2	7.7	8.4	7.9	8.5	8.2	8.2	3.2†	7.0
X.....	8.7	8.4	8.1	8.1	7.9	8.9	8.0	8.4	3.2†	6.7
XI.....	9.3	9.0	8.7	8.4	7.5	8.0	7.9	8.2	2.6†	6.9
XII.....	9.5	10.4	8.1	8.5	9.2	8.9	8.7	8.6	2.6†	8.1
Total.....	54.7	56.9	48.8	50.2	48.1	50.6	48.2	50.4	17.8†	42.2
SOIL II										
I.....	7.3	7.6	7.1	7.2	7.1	6.7	7.1	6.9	0.3	5.7
II.....	8.3	7.9	7.1	7.5	6.2	7.2	5.8	5.7	0.4	6.0
VI.....	7.7	7.2	7.6	7.0	6.5	7.2	6.6	6.9	0.3	5.6
VII.....	8.0	8.5	6.8	7.3	7.3	7.5	7.6	6.3	0.3	6.0
VIII.....	8.1	9.4	8.1	8.4	8.5	7.0	7.9	7.8	0.4	7.1
IX.....	9.5	8.5	8.0	8.0	7.9	7.9	8.3	8.9	0.7	7.6
Total.....	48.9	49.1	44.7	45.4	43.5	43.5	43.3	42.5	2.4	38.0

* N, naphthalene acetamide; P, phenylacetic acid.

† When watered on October 22, only $\frac{1}{2}$ of the usual amount of naphthalene acetamide was supplied.

then—just before use—the proper amount was added to the diluted nutrient solution to make up the desired concentrations of the respective substances. The concentrations used were 10^{-4} , 10^{-6} , 10^{-8} , 10^{-10} , and 0.

The crocks containing soil I were watered with nutrient containing the respective growth substances October 22, November 9, 16, 24, 30, December 7. Soil II was watered October 30, November 9, 16, 24, 30, and December 7. On December 15 the plants constituting any given sample were carefully cut off at the cotyledonary node near the surface of the soil, the plants placed in a paper sack and

dried rapidly to brittleness. Later each sample was again dried to constant weight at 100° C.

Table 1 is a record of all the samples. The results showed considerable variation within any given treatment, depending upon their particular location on the greenhouse benches. As a whole, the plants in soil I made more growth and had greater

TABLE 2

AUXIN CONTENT OF SOILS, EXPRESSED AS MICROGRAMS OF INDOLEACETIC-ACID
EQUIVALENTS PER KILOGRAM OF AIR-DRY SOIL

LOT	SUBSTANCE AND CONCENTRATION									
	0		10 ⁻¹⁰		10 ⁻⁸		10 ⁻⁶		10 ⁻⁴	
	CONTROL	CONTROL	N*	P*	N	P	N	P	N	P
SOIL I										
I.....	0.000	0.000	0.035	0.000	0.000	0.000	0.000	0.000	0.031†	0.000
II.....	0.029	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.020†	0.000
VI.....	0.042	0.000	0.000	0.039	0.063	0.000	0.000	0.000	0.140†
VII.....	0.035	0.128	0.000	0.126	0.000	0.051	0.000	0.093†	0.000
VIII.....	0.082	0.046	0.000	0.158	0.090	0.034	0.000	0.000	0.031†
IX.....	0.033	0.000	0.083	0.025	0.057	0.000	0.020	0.014	0.000†
Average....	0.032	0.029	0.032	0.058	0.030	0.006	0.017	0.002	0.045†	0.000
SOIL II										
III.....	0.000	0.038	0.000	0.114	0.000	0.000	0.000	0.155	0.000	0.000
IV.....	0.000	0.125	0.000	0.000	0.000	0.000	0.000	0.000
V.....	0.000	0.131	0.034	0.000	0.000	0.000	0.000	0.000	0.075
X.....	0.122	0.129	0.000	0.038	0.000	0.150	0.142	0.000	0.000	0.127
XI.....	0.024	0.000	0.000	0.000	0.118	0.104	0.000	0.000	0.076	0.000
XII.....	0.000	0.170	0.000	0.102	0.000	0.000	0.022	0.147	0.023	0.115
Average....	0.024	0.099	0.000	0.048	0.024	0.042	0.027	0.050	0.014	0.053

* N, naphthalene acetamide; P, phenylacetic acid.

† When watered on October 22, only $\frac{2}{3}$ of the usual amount of naphthalene acetamide was supplied.

dry weight than those in soil II, but with respect to their responses to the growth-regulating substances the trends in the two soils were similar. This trend clearly shows progressively less total dry weight with increasing concentration of the growth substance supplied, with a very abrupt decrease between 10⁻⁶ and 10⁻⁴. This decrease is much more marked in the case of the plants treated with naphthalene acetamide. None of the concentrations resulted in any increase in dry weight. Except for the fact that none of the results showed an increase, the results in gen-

eral closely approximated those obtained when quartz sand and a nutrient solution were used instead of soil. No measurements were made on root development.

After all the plants had been harvested, comparable samples of each random lot of soil were made up by combining aliquots from each of the pots in any given row. These samples were sent to Dr. WILLIAM S. STEWART at Beltsville, Maryland, who tested them according to his method of determining the amount of auxin in soils. The results of these determinations are given in table 2, the random samples being listed in the same sequence as they occur in table 1. At present no deductions are drawn from the data, other than the fact that the two growth-regulating substances seem to have had little or no effect, either in increasing or in decreasing the amounts of detectable auxin in the two soils. The figures are presented chiefly to show the wide range of variation in the results obtained; such variation may be partly inherent in the method of testing (3).

As stated, the results from this experiment are similar to those obtained when quartz sand and various nutrient solutions were used as a medium for growing the plants, instead of soil, except that in no instance were there increases in growth over that of the controls. Even the most dilute concentration, 10^{-10} , resulted in less growth. There are no clear, direct indications that the physical texture, the soluble salt content, or the quantity or type of organic matter present in these soils directly influence the general effects of these two growth substances when supplied to tomato plants. The results do not at present support the rather generalized claim that the growth of plants is greatly increased when small quantities of either of these substances is added to the soil.

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LITERATURE CITED

1. HAMNER, CHARLES L., Physiological and chemical responses of bean and tomato plants to alpha naphthalene acetamide and phenylacetic acid. *BOT. GAZ.* 103:374-385. 1941.
2. LAUDE, H. M., Combined effects of potassium supply and growth substances on plant development. *BOT. GAZ.* 103:155-167. 1941.
3. STEWART, W. S., and ANDERSON, M. S., Auxins in some American soils. *BOT. GAZ.* 103:570-575. 1942.
4. SWARTZ, DAPHNE B., Effect of various growth-regulating substances upon several species of plants. *BOT. GAZ.* 103:366-373. 1941.

RIBOFLAVIN IN ISOLATED ROOTS¹

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Riboflavin is not known to be an indispensable growth supplement for any isolated root whose continued cultivation *in vitro* has yet been reported. Although it is possible that roots requiring supplements of this substance may be found, still it is evident that an outside source of riboflavin is not required by roots of tomato, alfalfa, clover, and others which are known to require supplements of thiamin, nicotinic acid, and/or vitamin B₆. The data presented here suggest that riboflavin is synthesized by certain isolated roots.

Isolated roots were cultured according to methods reported earlier (1). The culture medium contained, per liter of redistilled water, 236 mg. Ca(NO₃)₂ · 4H₂O, 36 mg. MgSO₄ · 7H₂O, 81 mg. KNO₃, 65 mg. KCl, 20 mg. KH₂PO₄, 1.5 mg. ferric tartrate, and 20 gm. sucrose. Supplements of thiamin and vitamin B₆ were added in the concentration of 0.1 mg. per liter of nutrient and nicotinic acid at the rate of 0.5 mg. per liter of nutrient. Supplements of thiamin and nicotinic acid only were used for roots of alfalfa and clover; supplements of thiamin, vitamin B₆, and nicotinic acid were used for all the other species.

All roots were subcultured at weekly intervals by removal of a 1-cm. tip (from the principal axis of the root) to fresh medium. The roots of alfalfa, clover, *Datura*, and sunflower had been in culture for more than 1 year when the experiments were initiated.

Assays for riboflavin were done by the microbiological method of SNELL and STRONG (2), using *Lactobacillus casei* as the test organism. Growth was allowed to take place for 24 hours at 37° C. and was then measured in a Fisher electrophotometer. In each experiment a linear relationship between growth of the organism as measured in this way and riboflavin supplement obtained over the range 0.00 to 0.10 γ (micrograms) of riboflavin. Duplicate determinations on the same sample differed by less than 5 per cent.

Extraction of riboflavin was carried out by autoclaving two roots in 5 cc. of water at a pressure of 15 lb. for 15 minutes. The water was then decanted, made up to 5 cc., 5 cc. of basal medium added, and the riboflavin determination made on the sample. Where 1-cm. root tips were assayed the extraction was done similarly, except that ten (five each for sunflower and *Datura*) tips were used per 5 cc. of water.

¹ Report of work done with the co-operation of the Work Projects Administration, O.P. no. 165-1-07-172. This work was made possible in part by the support of Merck and Company.

Table 1 gives the results of a preliminary experiment which shows that the apparent riboflavin content of isolated tomato roots is independent of the amount of root extract used as a supplement for *Lactobacillus* in the riboflavin assay. The

TABLE 1

DETERMINATION OF RIBOFLAVIN IN DIFFERENT AMOUNTS OF EXTRACT OF ISOLATED TOMATO ROOTS. ALL PHOTOMETER READINGS (ARBITRARY UNITS) HAVE THE BLANK SUBTRACTED. ALL FIGURES ARE MEANS OF DUPLICATE DETERMINATIONS

SUPPLEMENT	PHOTOMETER READING	SUPPLEMENT	PHOTOMETER READING	CALCULATED AMOUNT OF B ₂ (MICROGRAMS)	RECOVERY OF ADDED B ₂	
					AMOUNT	PERCENTAGE
None.....	0.0	Extract of 1 root.....	7.6	0.0196/root
0.025 γ B ₂ ...	10.4	Extract of 2 roots.....	15.8	0.0203/root
0.05 γ B ₂ ...	18.6	Extract of 4 roots.....	30.5	0.0197/root
0.075 γ B ₂ ...	28.8	Extract of 1 root +0.05 γ B ₂ ...	26.1	0.0673	0.048	96
0.10 γ B ₂ ...	38.6	Extract of 2 roots +0.05 γ B ₂ ...	35.3	0.0910	0.050	100
		Extract of 4 roots +0.05 γ B ₂ ...	50.6	0.130	0.051	102

TABLE 2

CONCENTRATION OF RIBOFLAVIN IN ISOLATED TOMATO ROOTS OF VARIOUS AGES. ASSAYS ON INITIAL ROOT TIPS ALL MADE ON INDIVIDUAL LOTS OF 10 ROOTS. ASSAYS ON CULTURED ROOTS MADE ON LOTS OF 2 ROOTS

INITIAL ROOT TIPS (1 CM. LONG)			CULTURED ROOTS (WITHOUT TIP)		
EXPERIMENT NO.	WEEKS IN CULTURE	γ B ₂ /ROOT TIP	EXPERIMENT NO.	WEEKS IN CULTURE	γ B ₂ /ROOT
2	0	0.0024 .0024 .0018	2	8	0.015 .016 .018
3	0	.0014 .0019 .0016 .0028 .0013 .0014 Av.=0.0019			0.026 Av.=0.019
			3	13	0.016 .014 .018 Av.=0.016
			4	24	0.020 .020 .020 Av.=0.020

data also show that added riboflavin may be quantitatively determined in the presence of the extract of isolated tomato roots. It does not appear, therefore, that substances present in such roots interfere in any way with the results of the assay.

Experimental results

A clone of isolated tomato roots was established by the propagation of branch tips of a root derived from one individual seedling (San Jose Canner, of the California Packing Corporation) root tip. Seedling tomato root tips 1 cm. long and

TABLE 3
CONCENTRATION OF RIBOFLAVIN IN ISOLATED ROOTS OF CLOVER
AND ALFALFA. INITIAL ROOT TIP ASSAYS BASED ON 10 TIP
SAMPLES. ASSAYS OF CULTURED ROOTS BASED ON 2 ROOT
SAMPLES

ALFALFA		CLOVER	
SOURCE OF ROOT	γ B ₂ /ROOT	SOURCE OF ROOT	γ B ₂ /ROOT
Initial root tips	0.0008	Initial root tips	0.0003
	.0017		.0003
	.0011		.0014
	.0008		.0008
	.0011		0.0008
	0.0019		Av. 0.0007
	Av. 0.0012		
In culture 68 weeks	0.009	In culture 68 weeks	0.021
	.015		.018
	.014		.029
	0.009		.015
			.018
	Av. 0.012		.012
			0.014
			Av. 0.018
In culture 70 weeks	0.014	In culture 70 weeks	0.024
	.014		.020
	0.018		.024
	Av. 0.015		0.021
			Av. 0.022
In culture 75 weeks	0.014	In culture 75 weeks	0.011
	.016		.011
	.011		.009
	0.011		0.011
	Av. 0.013		Av. 0.0105

similar to that from which the clone was derived were assayed for total riboflavin content. A series of determinations are given in the first column of table 2. After the clone had been propagated through eight successive weekly transfers, approximately 125 roots—averaging 50 mm. in length—were available. These roots were transferred by removal of the apical 10 mm. to fresh medium. Eight of the remain-

ing bases were then assayed for total riboflavin content. The procedure was repeated after the thirteenth and the twenty-fourth week in culture. These results are given in the second column of table 2. It is evident that the base (without the apical 10 mm.) of one cultured root contains roughly ten times as much riboflavin as does a root tip similar to that from which the entire clone was derived. At the end of the eighth week in culture, therefore, the entire stock of this clone would

TABLE 4
CONCENTRATION OF RIBOFLAVIN IN ISOLATED ROOTS OF DATURA
AND SUNFLOWER. INITIAL ROOT TIP ASSAYS BASED ON 5 TIP
SAMPLES. ASSAYS OF CULTURED ROOTS BASED ON 2 ROOT
SAMPLES

DATURA		SUNFLOWER	
SOURCE OF ROOT	γ B ₂ /ROOT	SOURCE OF ROOT	γ B ₂ /ROOT
Initial root tips	0.0025	Initial root tips	0.0022
	.0025		.0034
	.0023		.0028
	0.0032		.0038
	<u>0.0032</u>		.0044
	Av. 0.0026		0.0038
			<u>0.0038</u>
			Av. 0.0034
In culture 63 weeks		In culture 70 weeks	
	0.0155		0.013
	0.0155		.010
	<u>0.0155</u>		0.008
	Av. 0.0155		<u>0.010</u>
			Av. 0.010
In culture 68 weeks		In culture 75 weeks	
	0.014		0.021
	.018		.022
	.016		0.022
	0.014		<u>0.022</u>
	<u>0.0155</u>		Av. 0.022

appear to have contained roughly 125×10 , or over 1000 times as much riboflavin as did the original root tip. This would seem to indicate a riboflavin synthesis by the particular clone of isolated tomato roots in question.

Experiments generally similar to that just described were carried out on other species of isolated roots. Table 3 gives data concerning alfalfa and white clover. The two stocks of isolated roots used were not clones, but each had been increased in number by the propagation of branch root tips. Seedling root tips for riboflavin assay were obtained from the same stock of seeds as was originally used for establishing the isolated root stocks. The bases (after removal of 10-mm. apical growing points) were first assayed after sixty-eight successive weekly transfers. In the

sixty-eighth transfer the alfalfa roots had grown an average of 137 mm. and the clover roots an average of 106 mm. It is evident from table 3 that the bases (without 1-cm. apical tips) of both species contain more riboflavin than 1-cm. seedling root tips similar to those from which the isolated roots derived. The data for the seventieth and seventy-fifth weeks of culture support this view.²

Table 4 gives data similar to that of table 3 but pertaining to isolated roots of *Datura* and sunflower. The *Datura* roots represented a clone while the sunflower roots came originally from thirty individual seedling root tips, although the stock had been multiplied by the cultivation of branch root tips. The *Datura* roots had grown an average of 78 mm. during the sixty-third week and the sunflower roots an average of 52.5 mm. during the seventieth week when the first assays of table 4 were made. In each case seedling root tips were obtained from the same stocks of seeds as those used for obtaining the isolated roots. Although the data of table 4 are not extensive, they do indicate that the bases of cultured *Datura* and sunflower roots contain more riboflavin than seedling root tips similar to those from which the isolated roots derived.

Summary

1. The riboflavin content of 1-cm. root tips from aseptically germinated seedlings of five different species has been determined. In each case a similar root tip or tips was cultured *in vitro*.
2. Riboflavin determinations were done on isolated roots from the stocks thus established. The roots used for assay were bases from which 1-cm. apical tips had been removed and represented samples of the root tissue produced during each weekly culture period. The number of such bases which arose, during culture, from one initial seedling root tip was large; in the case of *Datura*, for example, over 1000 were actually produced during 63 weeks of culture, and more could have been produced had every branch tip been cultured. With every species, each of these bases contained markedly more riboflavin than the initial root tip from which the root or clone was derived. This evidence suggests that in all the species of isolated roots investigated, a synthesis of riboflavin took place during culture.

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LITERATURE CITED

1. BONNER, JAMES, On the growth factor requirements of isolated roots. Amer. Jour. Bot. 27:692-701. 1940.
2. SNELL, E. E., and STRONG, F. M., A microbiological assay for riboflavin. Ind. Eng. Chem. Anal. Ed. 11:346-350. 1939.

² No explanation can be given for the apparent low riboflavin content of the 75-weeks clover roots. The roots were in good condition and had grown an average of 107 mm.

EFFECT OF MINERAL NUTRITION ON THE ASCORBIC-ACID CONTENT OF THE TOMATO

K. C. HAMNER,¹ C. B. LYON,² AND C. L. HAMNER³

(WITH THIRTEEN FIGURES)

Introduction

A review of the literature concerning the nutritive value of the tomato fruit (3) has indicated that tomatoes may contribute significant amounts of ascorbic acid and provitamin A to the human diet, and that the relative amount of these constituents may vary considerably. It is known that these variations may be partially correlated with differences in the hereditary constitution of the plant and with environmental relations during the growing season. This series of investigations has been designed to measure the influence of environmental factors on the nutritive value of the tomato, and no attempt is made to measure genetic influences.

The environmental factors may be arbitrarily classified as: (1) factors of the root environment which are influenced largely by soil conditions, that is, supply of nutrient elements to the roots, temperature, aeration, moisture supply, etc.; (2) factors of the top environment which are influenced largely by climatic conditions, that is, temperature, light intensity, light quality, length of photoperiod, relative humidity, etc.

In this paper, ascorbic acid is the nutritive quality considered. The investigations have emphasized variations in the growth and development of the tomato plant which result from differences in the supply of macronutrient elements in sand culture. Results of a few experiments which measure the influence of factors other than mineral nutrition are included. Experiments concerning the influence of microelement nutrition—as well as the effect of other individual environmental factors on nutritive value—are in progress, and constituents other than ascorbic acid are being considered.

Four separate experiments have been carried out, using the Bonny Best variety of tomato as the experimental material. The first two are concerned with the influence of wide variations in the supply of macronutrient elements to plants growing in sand culture upon the growth, fruit production, and ascorbic-acid content of the fruit. The third and fourth deal with the ascorbic-acid content of fruit produced by plants under similar conditions of nutrient supply but subjected to variations in other environmental conditions.

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Investigation

EXPERIMENT I

METHODS.—The method of preparing nutrient solutions was adapted from the technique described by C. L. HAMNER (2). Six stock solutions were prepared with nine salts, using concentrations as given in table 1. In so far as the major nutrient ions are concerned, each cation stock solution contains only one cation but all three anions. The concentration of anions in the cation stock solutions is constant. Conversely, each anion stock solution contains one anion but all three cations in comparable concentrations. The stock solutions were combined on the basis of

TABLE 1
IONIC CONCENTRATIONS OF NUTRIENT STOCK SOLUTIONS
IN MILLIEQUIVALENTS PER LITER*

SALT	IONIC CONCENTRATIONS OF INDIVIDUAL STOCK SOLUTIONS					
	K	Ca	Mg	NO ₃	H ₂ PO ₄	SO ₄
KNO ₃	12.0	4.5
KH ₂ PO ₄	4.5	4.5
K ₂ SO ₄	9.0	4.5
Ca(NO ₃) ₂	12.0	12.0
Ca(H ₂ PO ₄) ₂	4.5	12.0
CaSO ₄	9.0	12.0
Mg(NO ₃) ₂	12.0	9.0
MgHPO ₄	4.5†	9.0
MgSO ₄	9.0	9.0

* To each stock solution was added: 0.5 p.p.m. B as H₂BO₃, 0.5 p.p.m. Mn as MnCl₂, 0.05 p.p.m. Zn as ZnSO₄, 0.02 p.p.m. Cu as CuSO₄, and 5.0 p.p.m. Fe as FeC₂H₃O₇.

† H₂PO₄ was added to this solution to raise the concentration of the H₂PO₄ ion to the required strength.

ninths to secure a range of twenty-eight solutions of varying cation concentrations and twenty-eight solutions of varying anion concentrations (fig. 1). Thus the apical position of the cation triangle lacks two cations; each side of the triangle lacks one cation; and the center portion of the triangle is supplied with all three cations in varying proportions. All treatments in the cation triangle are supplied with anions in equal concentrations.

On August 15, 1940, several thousand commercial seeds were planted in pure quartz sand and watered with a complete nutrient solution (position 13, fig. 1) which had been diluted with distilled water in the ratio of 1:2. The seeds were planted in small crocks, fifteen per crock, and after germination the seedlings were thinned out, leaving two uniform seedlings in each pot. The plants were watered with the complete nutrient solution once each week and at other times during the week were supplied with small amounts of distilled water.

On September 12 the plants were 28 days old and approximately 4 inches high. At this time the seedlings were selected for uniformity and were transplanted

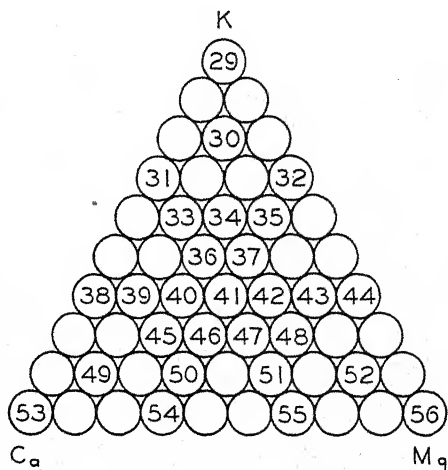
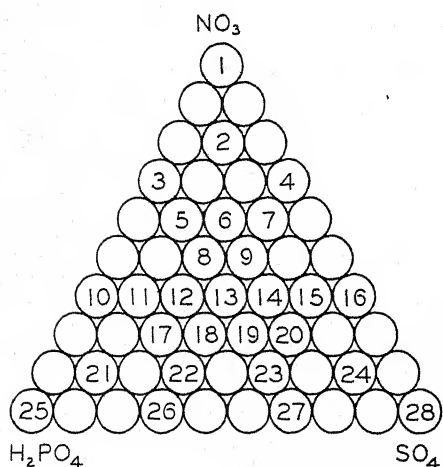
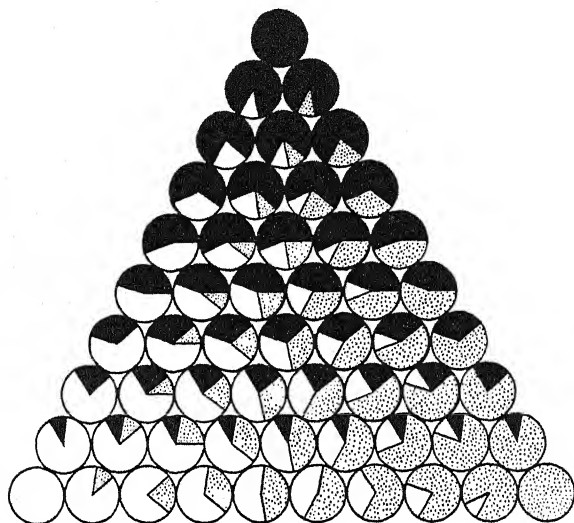


FIG. 1.—Upper triangle is a diagrammatic representation of relative proportions of stock solutions used to produce 55 possible combinations. Left, below: 28 combinations of anion stock solutions used in experiment I. Right, below: 28 combinations of cation stock solutions used in experiment I.

into 2-gallon glazed crocks containing pure quartz sand. Crocks totaling 1120 were used and each crock contained two seedlings. The design provided for fifty-six nutrient treatments with twenty crocks (forty plants) per treatment. The

treatments were randomized on the greenhouse bench to minimize the influence of environmental variations on the results. The plants were watered twice weekly with nutrient solution and were flushed thoroughly once a week with distilled water, prior to application of the nutrient. At other times the plants were watered with distilled water as needed. The greenhouse temperature was maintained as nearly as possible at 70° F., and low atmospheric humidities were avoided by frequently sprinkling the walks and walls of the house with water. Extreme care was exercised to preclude the possibility of infections with parasites or insects of any sort. The plants were pruned weekly of all axillary growth, and as flowers formed in the various treatments, the clusters were tapped by hand to insure pollination. A smaller amount of distilled water was used in watering the crocks as the light intensity decreased in the fall, but the plants were watered with the nutrient solution twice each week, a sufficient quantity being used to provide an appreciable amount of drip from each pot.

A portion of the plant population was harvested at 61 days, when many were in bloom. Fresh and dry weight data were obtained, and details on the appearance recorded. The remaining portion of the population was maintained on the respective nutrient treatments until sufficient ripe fruits were analyzed for ascorbic acid.

The chemical method was used in the determinations of ascorbic acid with a modification described by MORELL (6). The aliquot, however, was titrated to an end point with standardized dye. A transverse section weighing approximately 20 gm. was taken from the equatorial portion of the fruit. Various methods of sampling have shown that this procedure gives an accurate estimate of the average value of the fruit.

EXPERIMENTATION AND RESULTS.—On October 15 all the plants were 61 days old and had been supplied with their respective nutrient solutions for 33 days. Eight crocks (sixteen plants) in each treatment were harvested, and, in addition, the tops of one plant from each of the twelve remaining crocks in each treatment were removed. The dry weights of these plants were determined (fig. 2). The total of twenty-eight plants is used as a criterion in the estimation of growth in a particular nutrient treatment.

It may be seen that the several nutrient solutions employed have resulted in differing amounts of growth as measured by accumulation of dry weight. It therefore seems apparent that the variations in the nutrient combinations were of sufficient magnitude to affect the behavior of the plants, even at this early date. This conclusion is supported by the appearance of the plants at this time. Certain symptoms which developed on plants in various treatments could be correlated with the absence of one or more ions in the nutrient medium. In general, a treat-

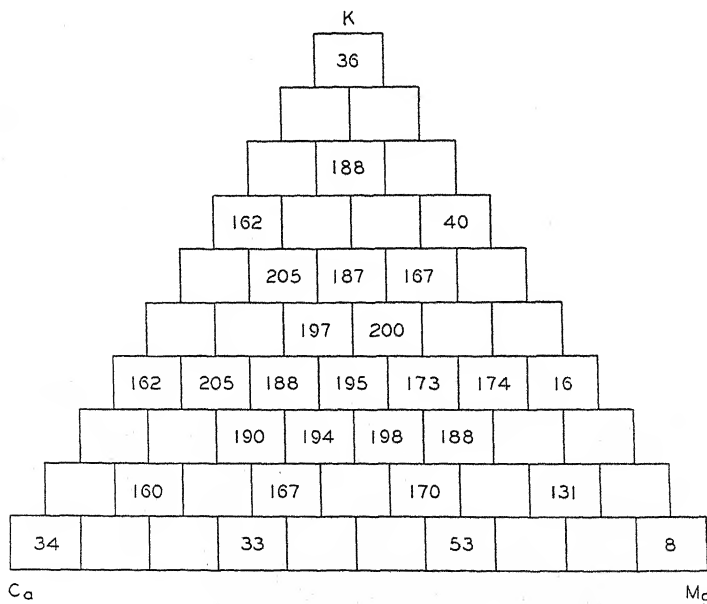
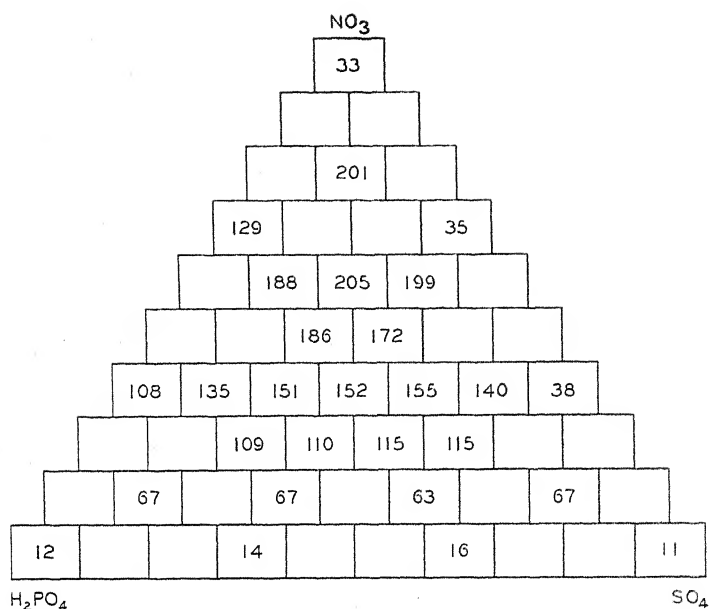


FIG. 2.*—Total dry weight in grams of the 28 vines of each treatment at first harvest of experiment I.

* In this and in all succeeding figures where data are presented, results are given in triangular positions corresponding to equivalent positions in figure 1.

ment deficient in two ions resulted in intermingling of symptoms characteristic of the respective single ion deficiencies and will not be discussed separately.

Phosphate-deficient treatments produced spindly plants with purple stems, deep purple-green upper leaves, and lower leaves which were pale green with a bronze cast. The dry weights were significantly less than the dry weights of plants produced under more optimum conditions of anion nutrition. The best appearance and growth in the phosphate-deficient treatments occurred in position 16, where relatively low nitrate and high sulphate conditions in the nutrient medium were prevalent.

Nitrate-deficient treatments produced small plants with pale yellow upper leaves. The lower leaves in general were dead or completely lacking. These symptoms were less striking in treatments with higher concentrations of nitrate and were not apparent under optimum conditions. Deficient treatments produced very little dry weight. With increasing nitrogen supply, the accumulation of dry matter became progressively greater. In the center portion of the triangle there is little or no indication of a sulphate or phosphate interaction.

In the two treatments when lack of sulphate was not accompanied by phosphate or nitrate deficiencies, sturdy light green plants with thin yellowish stems and chlorotic lower leaves were produced. The dry-weight accumulation was approximately 60 per cent of that produced under most optimum conditions of anion supply. When this deficiency was accompanied by the absence of another anion, the symptoms which developed more closely approximated those characteristic of the latter.

Of the plants in the cation triangle, those receiving solutions deficient in potassium were the smallest. The lower leaves were yellow, and brown spots developed on some of the younger leaves, with burning at the tips and margins of older leaves. A tendency for the leaves to curl downward was characteristic, especially with those solutions high in calcium and low in magnesium. Dry-weight accumulation of these plants was considerably less than in treatments represented in the center of the triangle.

In general, the plants grown in solutions deficient in calcium were small, with brown terminal buds which were in some cases dead, and in most cases the axillary buds turned brown and failed to develop. There were wide variations apparent in these treatments, and some of the plants were four or five times as tall as others. They grew vigorously, had fully expanded green leaves, and exhibited none of the symptoms of injury to the terminal bud. Occasionally one of the large plants was found growing in the same pot with another large plant, but more often they were found beside small plants showing extreme calcium deficiency. This variability in response to a nutrient supply deficient in calcium has been observed in other experiments. In this experiment, plants which were supplied with solutions con-

taining only magnesium as the cation were the smallest, and death of the terminal bud was not observed. In calcium-deficient treatments the accumulation of dry weight was relatively low.

At this time, plants grown in magnesium-deficient solutions which contained both calcium and potassium were light green, and the lower leaves were chlorotic. The dry-weight accumulation of these plants was approximately 80 per cent of the maximum obtained.

In both the anion and cation triangles, the treatments represented in the center portions produced healthy plants with no symptoms of chlorosis or necrotic areas on the leaves. The only apparent differences at this time were variations in size corresponding to those recorded in the dry-weight data.

Subsequent to the initial harvest, the remaining twelve crocks per treatment containing one plant per crock were redistributed and randomized in the greenhouse with enough space between them to provide the best available lighting conditions. The plants were continued on their respective nutrient treatments, and fruit started to ripen during the early part of December. Each fruit was picked on the morning of the day that complete color change had occurred. Immediately after removal, the fruit was weighed, an ascorbic-acid determination made on a representative sample, and the rest of the fruit prepared for analyses of other constituents. Only occasionally were two fruits removed from a single plant, and usually the fruit used represented the first one ripened. All determinations were made on the day that the fruit was picked, and the results of 318 analyses are given in figure 3. Within the limits of precision of this experiment, there are no significant differences in the ascorbic-acid content of fruit produced by different treatments. It is significant, however, that the average ascorbic-acid content of all the fruits that were analyzed (13.3 mg. per 100 gm. fresh fruit) was approximately 50 per cent of the average values reported in the literature (3). These tomatoes were produced in the greenhouse in the fall of the year, when low light intensities were prevalent. The data of other experiments have a direct bearing on this point.

The plants were continued on their respective nutrient treatments until December 19, 1940. At this time all remaining plants were harvested, and measurements of dry weight of root systems, dry weight of vine, and fruit production were recorded. It was noted that the dry weights as well as the quantity of fruit produced by the plants subjected to the various treatments were affected by relatively small variations in composition of the nutrient solutions. Variations occurred as the result of differences represented in the central region of the triangles. The symptoms characteristic of deficiencies were also apparent in treatments containing small amounts of certain ions. Since the trends shown in data for these characters closely coincide with results obtained in experiment II, they are discussed later.

Figure 1 shows the crystal structure of the NO_3^- ion. The structure is a trigonal bipyramid with a central nitrogen atom (N) and six oxygen atoms (O) at the vertices. The N-O bond length is 137 pm, and the O-N-O bond angle is 120 degrees. The diagram is labeled with NO_3 and SO_4 .

[illegible]

EXPERIMENT II

Since the results of experiment I indicated that the ascorbic-acid content of the tomato fruit is not affected to any appreciable extent by wide variations in the supply of macronutrient elements to the plant, and since all values were relatively low, it seemed desirable to repeat the experiment during the regular growing season and to grow the plants outside instead of in the greenhouse. Several modifications of experiment I were introduced, including the use of an inbred strain of tomatoes, the use of more nutrient combinations, the analysis of a greater number

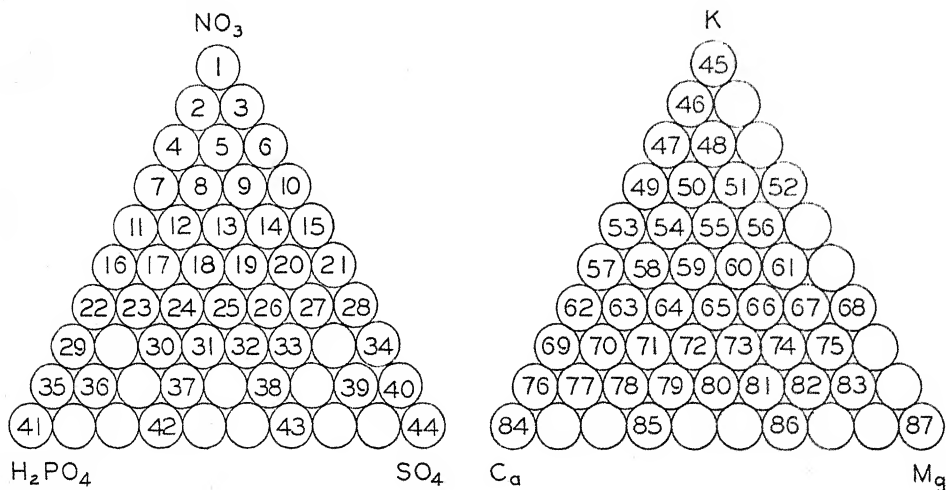


FIG. 4.—Left, the 44 combinations of anion stock solutions used in experiment II and right, the 43 cation combinations. Refer to figure 1 (top) for proportions of stock solutions in each combination.

of fruits for ascorbic acid, and a specific design for reduction and analysis of the data by statistical methods.

METHODS.—The inbred strain of the Bonny Best tomatoes used has been inbred for seven generations.⁴ Seed was planted in the greenhouse on May 12, 1941, in 2-quart glazed crocks containing pure quartz sand. Two seeds were planted in each crock, and a complete nutrient solution (see treatment 25, fig. 4) was used during germination and seedling stage. On June 9, when the seedlings were 28 days old and approximately 3 inches tall, uniform seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand, one seedling per crock. The seedlings were watered in with distilled water and immediately supplied with their respective nutrient treatments. On July 1, all plants were placed outdoors, the crocks being supported on wooden blocks. The plants were trained upright,

⁴ Seed supplied through the courtesy of Dr. LeRoy Powers, Senior Geneticist, U.S. Horticultural Field Station, Cheyenne, Wyoming.

and both crocks and plants were supported by stakes. All axillary growth was pruned off twice weekly, and the plants were harvested September 10.

The design of the experiment was that of a randomized block (1) with eighty-seven treatments and four replications. Each replication consisted of a three-plant row and was randomized by the use of TIPPETT's tables (8). Thus the mean of twelve plants was used as an estimation of the response to a given treatment, and a total population of 1044 plants was used. The data were reduced by means of the analysis of variance, and the *t* test (7) was used for determining whether particular differences were significant. Odds as great as or greater than 99:1 against the deviations noted, being due to the errors of random sampling, were accepted as statistically significant. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained both in the greenhouse and in the field.

Nutrient stock solutions were made up exactly as in experiment I, and the system of triangulation was the same. The number and position of the treatments, however, were modified (fig. 4). Thus, in this experiment, the nutrient stock solutions were combined on the basis of ninths to provide forty-four solutions of varying anion composition and forty-three of varying cation composition.

During the early growth stages, nutrient solution was supplied to the plants three times each week. As the summer progressed, nutrients were supplied every other day, and finally—when fruits were ripening—they were applied four times per week. The solutions were applied in quantities sufficient to insure an appreciable amount of drip, and each pot was watered with distilled water between nutrient applications.

During germination and the seedling stage, greenhouse temperatures were maintained at 85° F. in the daytime and 70° at night. Subsequent to transplanting, the temperatures in the greenhouse closely approximated those prevailing outside.

The mean daily temperature for the duration of the experiment was 67.7° F., with a mean maximum of 80.9° and a mean minimum of 55.3° F. The mean maximum temperatures in June, July, and August were 79.8°, 84.6°, and 79.3° F., respectively. Mean relative humidity from May 12 to September 10 was 45.2 per cent at 1:30 P.M. The average daily total of solar radiation for this period was 520.7 gram calories per square centimeter of horizontal surface.

EXPERIMENTATION AND RESULTS.—On July 23, all plants were 72 days old and had received their respective nutrient solutions for 46 days. At this time—and at weekly intervals thereafter—each plant was measured and the height in centimeters recorded. When the plants were 120 days old and had received their respective nutrient treatments for 94 days (September 9), they were harvested, the vines immediately weighed, and the number of elongated internodes counted.

The root systems were washed as free from sand as possible. Subsequent to this time, the vines and root systems were dried at 60° C. for 4 days in a forced-draft oven. The root systems were separated from all sand particles by pulverizing the dried material with adhering sand and separating these components by air pressure. Both the root systems and the vines of each plant were weighed and the dry weights recorded. These data were compiled, and the average length of internodes, the percentage dry matter of the vine, and the top-root ratio computed.

Fruit started to ripen in late July and early August. On July 25, data were recorded on the occurrence of blossom-end rot. These data, together with the results of mineral analyses on the fruit, are reported in a separate paper (5). All diseased fruits were discarded. Only healthy normal fruits were allowed to mature, and each fruit was picked on the morning of the day that complete color change had occurred. The entire population was examined daily until the end of the experiment. When picked, a fruit was immediately weighed, a portion analyzed for ascorbic acid, and the remaining portions prepared for analyses of other constituents. Only the first two fruits which ripened per plant were used for analytical procedures, but records were kept of the weight of every fruit as it was picked in order to compile data on total fruit production and mean fruit size. Upon completion of the experiment, all fruits were picked, counted, and weighed.

During the course of the experiment, careful notations were made on the gross appearance of the plants. The differences in the various treatments were similar to those recorded in experiment I and are not given in detail here. However, certain contrasts were obvious. The lack of uniformity in plants supplied with calcium-deficient nutrients noted in experiment I was not apparent in experiment II. Symptoms of calcium deficiency developed shortly after initiation of the treatments, and death of the terminal buds occurred within 32 days. Responses to low nitrate concentrations were more striking in the second experiment than in corresponding treatments of the first. Maximum growth occurred in comparable treatments in both experiments; but the plants were more sturdy, had thicker stems, produced more and larger fruit, and did not grow so tall in the second. The differences in environmental conditions of the two seasons represented should be kept in mind. It is, however, the similarities in response to treatment which should be emphasized. That the general trends of experiment I are duplicated in experiment II is interpreted as evidence that they represent actual responses on the part of the plant to variations in nutrient composition.

The results of the growth measurements are given in figures 5 and 6. The curves are presented largely for comparison and for an estimate of the relative rates of growth at different times. Data on growth and fruitfulness are given in figures 7-11.

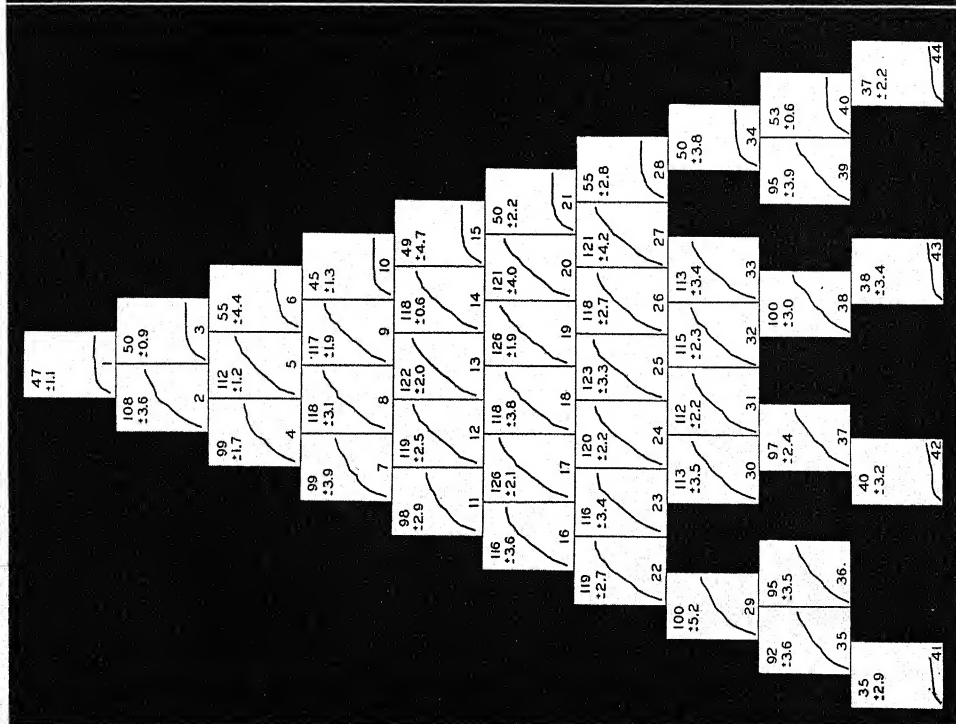


FIG. 5

FIG. 5.—Graph of average weekly height measurements of plants in anion treatments in experiment II. Data above each curve represent mean height in centimeters together with its standard error. Treatment numbers indicated below each curve. Fig. 6 (right), same as figure 5, except that cation treatments are presented.

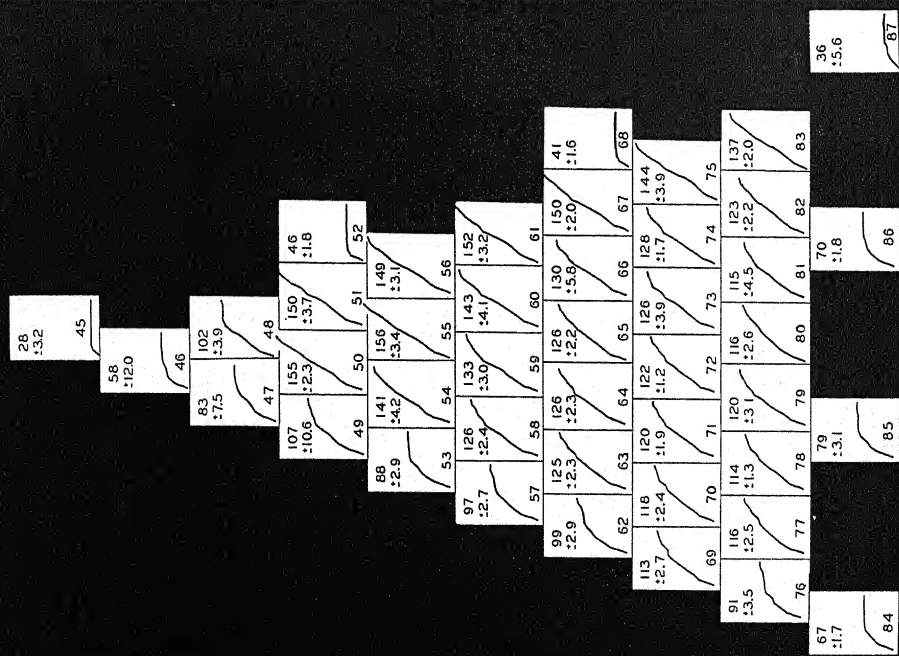


FIG. 6

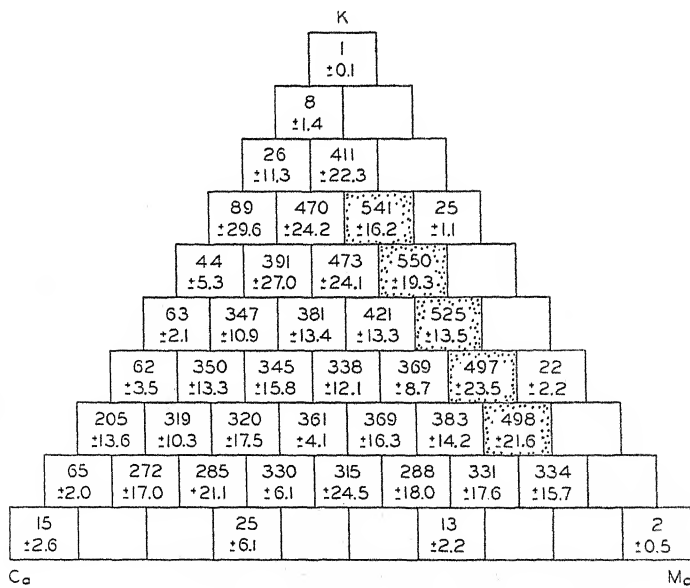
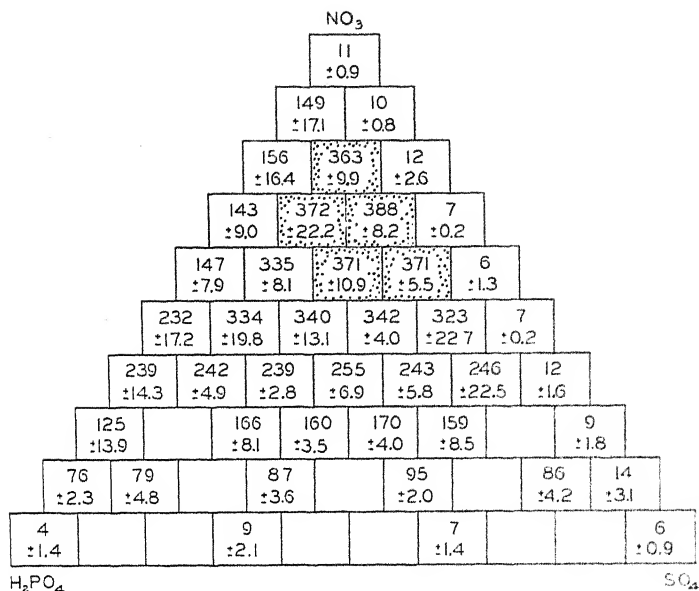


FIG. 7.*—Fresh weight of vine (in grams) for anion treatments (above) and cation treatments (below) of experiment II.

*In figs. 7-12, treatment means given together with their standard error. In any inter-treatment comparison, six degrees of freedom are available. When $t = 3.71$, $P = 0.01$.

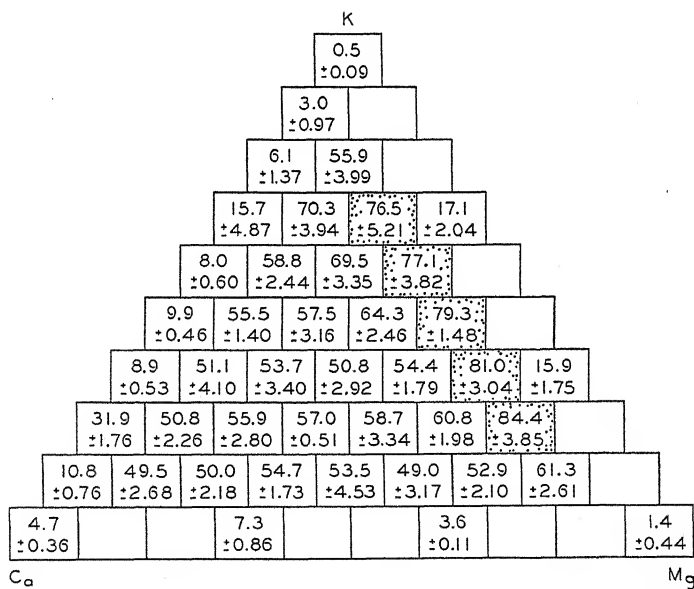
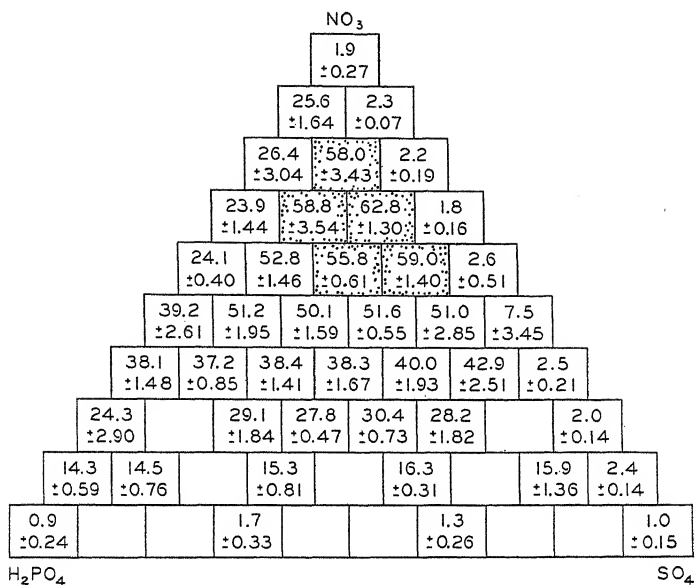


FIG. 8.—Dry weight of vine (in grams) for anion treatments (above) and cation treatments (below) of experiment II.

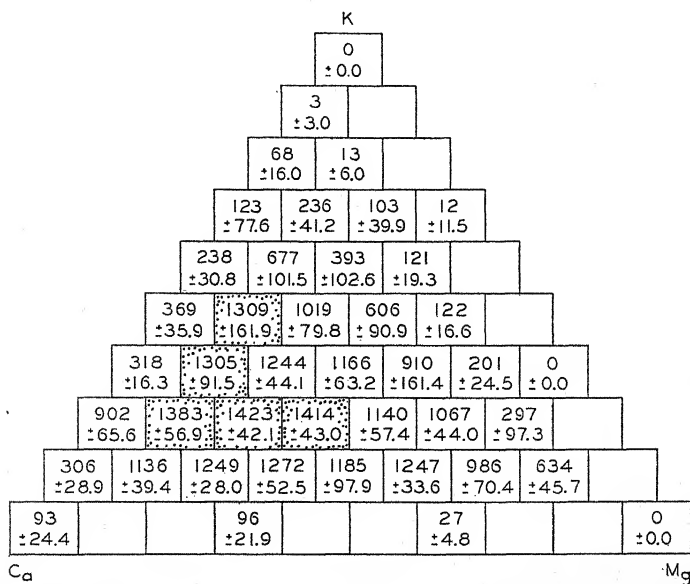
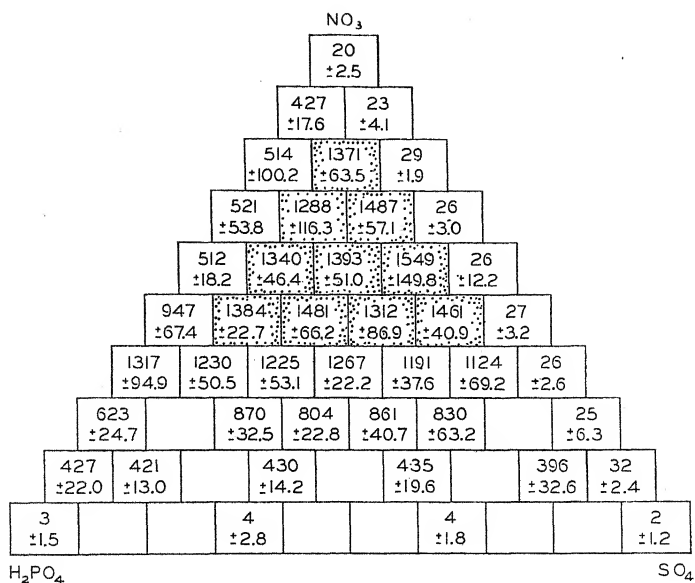


FIG. 10.—Total fresh weight of fruit per vine (grams) for anion treatments (above) and cation treatments (below) of experiment II.

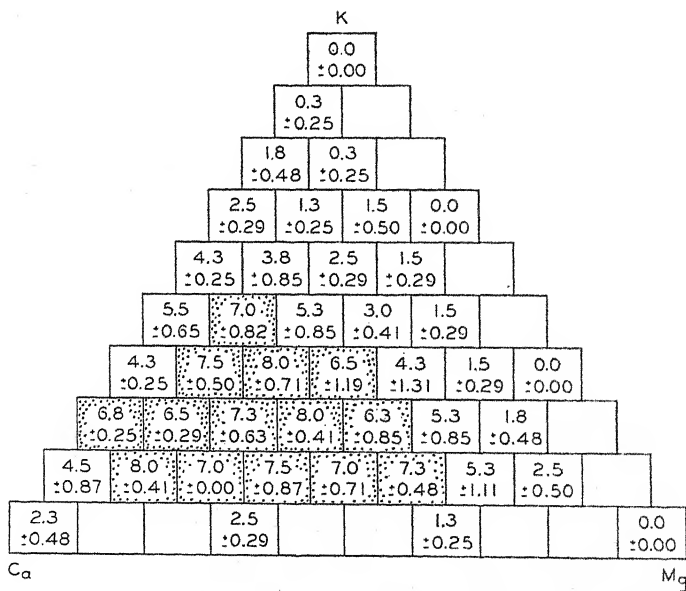
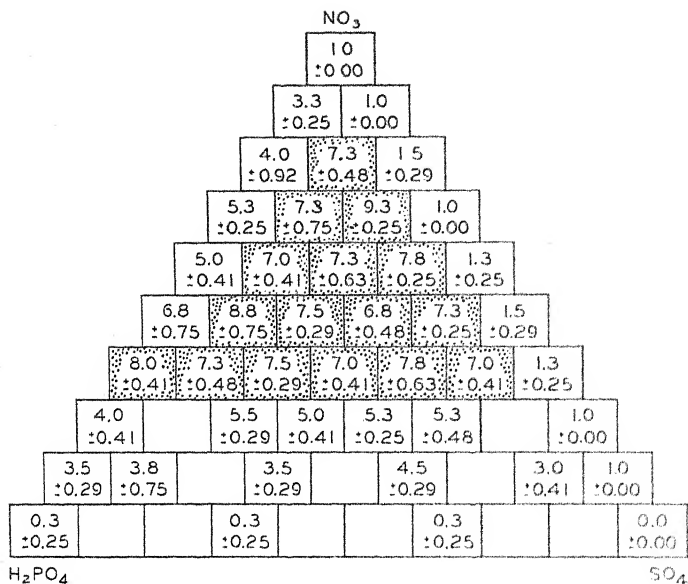


FIG. 11.—Number of fruit which ripened per plant for anion treatments (above) and cation treatments (below) of experiment II.

This experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences. Accordingly, analyses of variance were computed for each individual character, and the results are compiled in table 2. Since one replication in one

TABLE 2
RESULTS OF ANALYSES OF VARIANCE ON CHARACTERS
FOR WHICH DATA WERE RECORDED

CHARACTER	F VALUE	
	BETWEEN TREATMENTS*	BETWEEN REPLICATIONS†
A. Vegetative growth		
1. Height of plants (weekly measurements).....	119.71	14.89
2. Number of internodes.....	71.99	0.08
3. Average length of internodes.....	14.44	26.58
4. Fresh weight of vine.....	170.88	2.54
5. Dry weight of vine.....	133.70	4.87
6. Percentage dry matter of vine.....	22.77	21.72
7. Dry weight of root system.....	5.32	2.60
8. Top-root ratio.....	10.02	7.73
B. Fruit production‡		
1. Total fresh weight of fruit produced.....	101.51	1.70
2. Total number of fruit produced.....	60.03	1.21
3. Average fresh weight of fruit (regardless of state of maturity).....	44.62	1.51
4. Mean number of fruits which ripened per plant....	31.85	5.50
5. Average fresh weight of mature fruit.....	33.45	0.85
6. Number of days from planting to date of first fruit ripe.....	11.91	0.79
C. Vitamin C content of first two mature fruits on each plant.....	5.47	5.27

* An F value of 1.34 is required for a P value of 0.05; when $F = 1.49$, $P = 0.01$.

† An F value of 2.64 is required for a P value of 0.05; when $F = 3.86$, $P = 0.01$.

‡ All fruits infected with blossom-end rot discarded from this experiment.

treatment was discarded as a result of mechanical injury, YATES' formula (9) was used for the estimation of the missing plot in order to maintain orthogonality. Thus the sources of variations are:

VARIAION DUE TO:	NO. OF DEGREES OF FREEDOM
Treatments.....	86
Replications.....	3
Treatments×replications (error).....	257
Total.....	346

This procedure was followed for all characters except height of the plants. In this character the data were further reduced in order to furnish additional information.

It is evident from table 2 that treatments produced statistically significant differences in all the characters listed. It is also evident that differences in environment—when measured by replications—produced significant differences in such characters as height of plant, average length of internodes, dry weight and percentage dry matter of the vine, top-root ratio, mean number of fruits ripening per plant, and ascorbic-acid content of the fruits. This means that replication and randomization were essential to the design of the experiment. Since replication differences were measurable in the analysis of variance, and since these differences were not included in inter-treatment comparisons, valid differences exist which can be ascribed directly to the treatments.

The analysis of variance for height of plants provides one other point which is pertinent to the interpretation of results. In this character, where 12,528 measurements were involved, the validity of a treatment \times replication interaction was tested. A significant F value of 1.41 (when $F = 1.23$, $P = 0.01$) was obtained for this source of variation, which means that treatments do not react equally to differences in environment.

The data obtained in the anion treatments show uniform trends in all the characters present. The shaded portions of the triangles represent those treatments where maximum growth or fruitfulness was obtained. It is evident that, in general, the same treatments fall in the shaded portion for every character. These treatments contain 14.2–17.0 milliequivalents of nitrate per liter of nutrient medium. At lower concentrations of nitrate growth and fruitfulness were correspondingly less. In the nitrate-deficient treatments little growth was obtained after 32 days (fig. 5). However, some growth subsequent to this time is shown in the growth curves, which may be indicative of nitrogen reutilization.

When phosphate was absent in the nutrient media, growth and fruitfulness were poor. In this experiment it is evident that 2.8 milliequivalents of phosphate (one-ninth of the phosphate stock solution) was sufficient to result in a maximum expression of any character under conditions of comparatively high nitrate and low sulphate supply. Plants grown in sulphate-deficient treatments were the most productive of any grown in the anion deficiencies. In treatment 22, the plants were as tall as any in the anion triangle, and mean values for other characters were 60–100 per cent of maximum. Treatments where maximum values were obtained for all characters (shaded areas) contained 2.8–8.5 milliequivalents of sulphate.

Since growth and fruitfulness were limited by nitrate supply in most of the treatments, the effects of the higher concentrations of sulphate and phosphate may have been concealed. In sulphate-deficient treatments the higher nitrate and low phosphate concentrations were detrimental. This does not conform to the general effect of nitrate supply and will be emphasized in the discussion of

ascorbic-acid values. Treatments 9, 13, and 14 give maximum values for all characters, while certain other treatments have resulted in comparable values for some of the characters and not for others. Thus, in treatment 22, maximum fruitfulness and growth in height were attained, but fresh and dry weights of vines were significantly less. The sulphate-deficiency symptoms which occurred in these plants give supporting evidence that treatment 22 was not optimum. When many characters are evaluated, it is possible to demonstrate statistically significant differences for small variations in ionic concentrations of the nutrient medium. It is also possible—within relatively narrow limits—to determine the optimum composition of the solutions used.

The interpretation of treatment effect in the cation triangle depends upon the character under consideration. In general, variations of growth in height among treatments followed the same trends as were obtained for fresh weight of vines and dry weight of vines and roots. This group of characters is therefore discussed as a unit and contrasted with fruitfulness where different trends are apparent.

Calcium deficiencies resulted in the smallest plants produced in any of the cation treatments. When as small an amount as 2.8 milliequivalents of calcium per liter was present in the nutrient medium (shaded areas), growth in height and fresh and dry weights were maximum. This is the lowest concentration used in any treatment containing calcium. The concentration of potassium and magnesium in optimum treatments varied from 5.7 to 17.0 milliequivalents per liter, and variations in their relative proportions produced no significant effects within these ranges. The effect of treatment on the dry weight of the roots is discussed in connection with a consideration of the percentage dry matter. In potassium-deficient treatments—unless calcium was absent—growth was approximately 40 per cent of maximum when height of plant was used as a criterion. The fresh weight of vines and dry weights of vines and roots were relatively less, with no values exceeding 10 per cent of maximum. With magnesium deficiencies, certain treatments produced much more growth than others, depending upon the relative proportions of calcium and potassium present. Plants of treatment 69 were 80 per cent as tall and produced 30–40 per cent as much fresh and dry weight as the optimum treatments. It may be noted that treatments deficient in both potassium and magnesium resulted in relatively tall plants with comparatively little fresh or dry weight.

The data for fruitfulness may be contrasted with the observations for other characters. Nutrient treatments resulting in maximum values for total weight of fruit per vine contained the following milliequivalents per liter of major cations: 11.3–17.0 of calcium, 2.8–8.5 of magnesium, and 5.7–11.3 of potassium. The greatest number of fruits ripening per plant occurred over a wider range of concentrations but include the preceding optimum treatments. Other data, including

number and average weight of fruit produced, support the generalization that fruit production is greatest in high calcium treatments. As the calcium content of the nutrient medium was less, fruitfulness was correspondingly less; but at low concentrations of calcium, fruit production was greater in those treatments containing relatively low potassium and high magnesium. It is apparent that plants grown in treatment 69 (deficient in magnesium) were approximately 70 per cent as fruitful as any in the cation triangle, and it will be recalled that these plants also produced relatively large vines. Optimum fruitfulness and optimum vine growth have not occurred in the same nutrients. However, total dry weight production (vine+roots+fruit) was greatest in treatments where best fruitfulness was observed.

It is evident from table 2 that detailed data for many characters are not presented. However, the data for percentage dry matter in vines furnish several pertinent correlations and are given in figure 12. In general, there is an inverse correlation between growth and fruitfulness and percentage dry matter. Significantly high values for the latter character were obtained in all deficiency treatments (except in some deficient in sulphate and in one deficient in magnesium). In treatment 48, the percentage dry matter of the vines was significantly less than in any other treatment. Figure 9 shows that maximum dry-weight accumulation in root systems also occurred here. There is no obvious explanation for this correlation.

Certain correlation coefficients contribute to the interpretation of the data. From table 2 it is apparent that statistically significant differences between treatments were demonstrable for the average length of internodes as well as for the number of internodes. The average length of internodes was significantly less when plants were grown in nitrate-deficient solutions and was significantly greater in treatments deficient in potassium (except the treatment also deficient in calcium) and in those treatments where maximum growth in height occurred (50, 51, 55, 56, 61, 67, 75). The greatest number of internodes coincided with the greatest height of plants in the anion triangle; but such was not the case in the cation triangle, where maximum values were obtained in treatments 50, 54, and 55. Further reduction of the data therefore seemed desirable. Assuming x =height of the plant, y =number of elongated internodes, and z =average length of elongated internodes, then

$$\begin{aligned} (a) \quad r^{xy} &= +0.977 \\ (b) \quad r^{xz} &= +0.268. \end{aligned}$$

With the respective number of degrees of freedom involved (85), a value of 0.275 is required when $P=0.01$, and thus a significant correlation exists in equation (a) and is not demonstrable in equation (b). According to P values arbitrarily re-

⁵ r , Correlation coefficient between indicated variables.

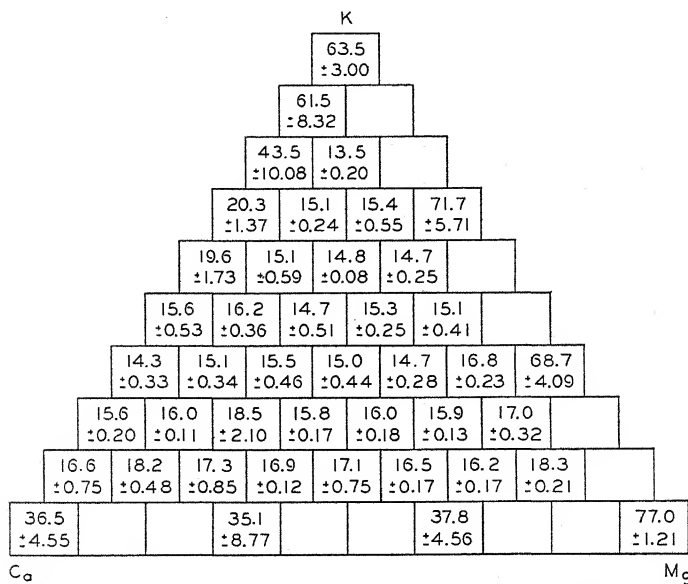
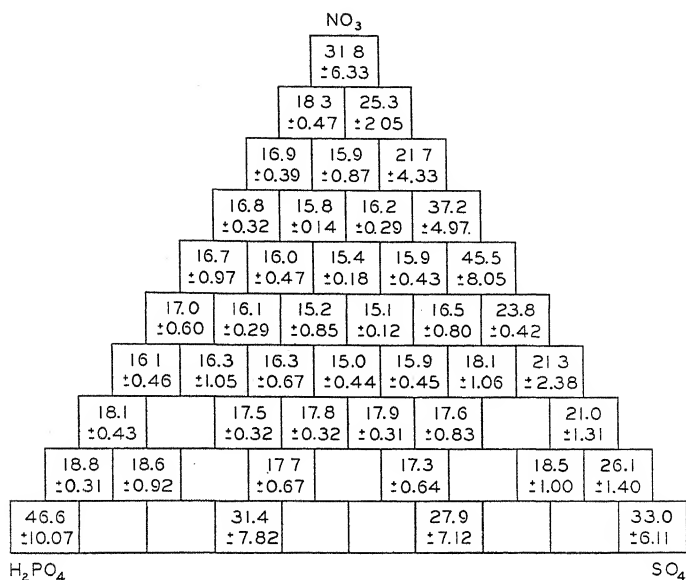


FIG. 12.—Percentage dry matter of vines for anion treatments (above) and cation treatments (below) of experiment II.

quired in this experiment, no general correlation occurred between height of plant and average length of elongated internode, while a highly significant correlation occurred between height of plant and number of internodes. This is reasonable considering the variation in response obtained, and it is surprising that significantly longer internodes occurred in certain treatments.

Data on top-root ratio, total number and average weight of fruit, and earliness support in general the other observations that growth and fruitfulness are significantly affected by the variations in nutrient composition in this experiment. It is felt that individual discussion of these characters is not necessary.

Ascorbic-acid determinations were made on 1447 fruits in this experiment, and the results are presented in figure 13. From table 2 it is evident that statistically significant differences between treatments occur for this character. However, no significant differences were obtained between any of the treatments not deficient in one or more of the macronutrient elements. Deficiencies in potassium and phosphate resulted in significantly less ascorbic-acid content, while treatments deficient in nitrate resulted in relatively high values but few fruit were produced, and the results are not statistically different from the other treatments. No fruits were analyzed in treatments deficient in calcium, since none matured. Significantly higher ascorbic-acid values were obtained in treatments 2, 4, 7, and 11, which were deficient in sulphate. These treatments were relatively high in nitrate and low in phosphate, and it was pointed out in the discussion of fruitfulness that such a condition was detrimental.

Although significant mean differences in ascorbic-acid values are demonstrable, the magnitude of the differences is not great. These conditions of extreme nutrient deficiency are probably rarely encountered in commercial production. The important fact is indicated that a macronutrient supply adequate to produce a fair yield is not a limiting factor in ascorbic-acid content. In situations where extreme deficiencies of potassium and phosphate seriously limit production, fertilization may increase ascorbic-acid content as well as yield.

While many data obtained in experiment I have not been included in this paper, it should be emphasized that the same trends were apparent in both experiments. The maximum values for growth and fruitfulness occurred with identical conditions of nutrient composition, and—as in experiment II—the highest ascorbic-acid value in experiment I resulted from sulphate deficiency. In the latter experiment, however, the plants produced more fresh and dry weight and were far more fruitful. Average ascorbic-acid values were also twice as high.

EXPERIMENT III

In spite of the fact that the ascorbic-acid content of the fruits from various treatments was remarkably uniform in each of the previous experiments, a wide

NO ₃			PO ₄			SO ₄			Ca			Mg		
19.3 ±1.75 [6]	31.7 ±1.02 [2]	21.6 ±1.90 [9]	27.2 ±1.44 [18]	28.5 ±0.96 [23]	29.0 ±0.94 [21]	29.0 ±0.96 [23]	30.0 ±6.04 [4]	30.0 ±1.55 [2]	21.9 ±1.72 [13]	19.0 ±1.01 [10]	19.8 ±1.57 [11]	25.3 ±1.14 [16]	25.9 ±1.11 [19]	25.3 ±0.81 [19]
29.3 ±0.86 [18]	26.7 ±1.16 [18]	22.3 ±0.95 [11]	27.1 ±1.45 [21]	27.6 ±1.36 [24]	28.9 ±1.30 [21]	29.6 ±1.54 [22]	29.1 ±3.36 [4]	36.1 ±1.55 [2]	27.4 ±0.94 [21]	25.5 ±1.15 [22]	26.7 ±1.18 [21]	27.5 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
30.9 ±0.85 [24]	24.0 ±1.18 [23]	26.0 ±1.44 [21]	27.1 ±1.48 [20]	27.4 ±0.88 [22]	29.0 ±1.58 [19]	29.6 ±1.52 [20]	32.8 ±3.18 [3]	36.1 ±1.55 [2]	25.9 ±1.28 [18]	28.5 ±1.06 [22]	28.4 ±0.82 [21]	27.8 ±1.71 [13]	28.6 ±1.12 [20]	28.6 ±1.12 [20]
32.4 ±0.95 [24]	24.8 ±1.28 [22]	25.2 ±1.16 [19]	25.7 ±1.14 [19]	20.5 ±2.94 [10]	21.5 ±1.02 [12]	23.2 ±2.11 [10]	23.8 ±2.17 [18]	23.8 ±2.17 [18]	22.2 ±0.90 [18]	27.7 ±1.45 [19]	25.2 ±1.24 [23]	26.2 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
23.8 ±2.17 [18]	25.0 ±0.99 [22]	27.1 ±1.45 [21]	27.7 ±1.48 [20]	21.5 ±1.02 [12]	21.5 ±1.02 [12]	23.2 ±2.11 [10]	23.8 ±2.17 [18]	23.8 ±2.17 [18]	22.2 ±0.90 [18]	27.7 ±1.45 [19]	25.2 ±1.24 [23]	26.2 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
28.0 ±1.29 [21]	24.0 ±0.91 [22]	27.4 ±0.88 [22]	29.0 ±1.58 [19]	21.5 ±1.02 [12]	21.5 ±1.02 [12]	23.2 ±2.11 [10]	23.8 ±2.17 [18]	23.8 ±2.17 [18]	22.2 ±0.90 [18]	27.7 ±1.45 [19]	25.2 ±1.24 [23]	26.2 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
27.2 ±1.44 [18]	28.5 ±0.96 [23]	29.0 ±0.94 [21]	29.6 ±1.54 [22]	29.6 ±1.54 [22]	29.6 ±1.54 [22]	29.6 ±1.54 [22]	29.1 ±3.36 [4]	36.1 ±1.55 [2]	27.4 ±0.94 [21]	25.5 ±1.15 [22]	26.7 ±1.18 [21]	27.5 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
29.0 ±0.96 [23]	28.5 ±0.96 [23]	29.0 ±0.94 [21]	29.6 ±1.54 [22]	29.6 ±1.54 [22]	29.6 ±1.54 [22]	29.6 ±1.54 [22]	29.1 ±3.36 [4]	36.1 ±1.55 [2]	27.4 ±0.94 [21]	25.5 ±1.15 [22]	26.7 ±1.18 [21]	27.5 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
30.0 ±6.04 [4]	30.0 ±1.55 [2]	36.1 ±1.55 [2]	32.8 ±3.18 [3]	36.1 ±1.55 [2]	36.1 ±1.55 [2]	36.1 ±1.55 [2]	29.1 ±3.36 [4]	36.1 ±1.55 [2]	27.4 ±0.94 [21]	25.5 ±1.15 [22]	26.7 ±1.18 [21]	27.5 ±1.00 [21]	27.2 ±1.30 [14]	27.2 ±1.30 [14]
K			Ca			Mg			Mg			Mg		
20.1 ±7.27 [3]	25.8 ±1.86 [17]	23.0 ±0.99 [3]	25.3 ±1.01 [21]	28.6 ±1.05 [14]	27.8 ±1.57 [16]	25.7 ±1.58 [13]	25.4 ±1.62 [21]	29.0 ±1.96 [19]	25.9 ±1.34 [22]	28.5 ±1.06 [22]	28.4 ±0.82 [21]	27.8 ±1.12 [20]	28.6 ±1.71 [13]	28.6 ±1.71 [13]
20.1 ±7.27 [3]	25.8 ±1.86 [17]	23.0 ±0.99 [3]	25.3 ±1.01 [21]	28.6 ±1.05 [14]	27.8 ±1.57 [16]	25.7 ±1.58 [13]	25.4 ±1.62 [21]	29.0 ±1.96 [19]	25.9 ±1.34 [22]	28.5 ±1.06 [22]	28.4 ±0.82 [21]	27.8 ±1.12 [20]	28.6 ±1.71 [13]	28.6 ±1.71 [13]
20.1 ±7.27 [3]	25.8 ±1.86 [17]	23.0 ±0.99 [3]	25.3 ±1.01 [21]	28.6 ±1.05 [14]	27.8 ±1.57 [16]	25.7 ±1.58 [13]	25.4 ±1.62 [21]	29.0 ±1.96 [19]	25.9 ±1.34 [22]	28.5 ±1.06 [22]	28.4 ±0.82 [21]	27.8 ±1.12 [20]	28.6 ±1.71 [13]	28.6 ±1.71 [13]
20.1 ±7.27 [3]	25.8 ±1.86 [17]	23.0 ±0.99 [3]	25.3 ±1.01 [21]	28.6 ±1.05 [14]	27.8 ±1.57 [16]	25.7 ±1.58 [13]	25.4 ±1.62 [21]	29.0 ±1.96 [19]	25.9 ±1.34 [22]	28.5 ±1.06 [22]	28.4 ±0.82 [21]	27.8 ±1.12 [20]	28.6 ±1.71 [13]	28.6 ±1.71 [13]

Fig. 13.—Ascorbic-acid values in experiment II, giving treatment means together with their standard errors. Number of analyses in each treatment is recorded in brackets. Left, anion triangle and right, cation triangle.

variation was noted between the average values of the two experiments. Since the first experiment was conducted in the greenhouse during the fall and winter months and the second was out-of-doors during the summer, it seemed possible that climatic environmental factors might have been primarily responsible. The purpose of experiment III was to measure the influence of soils and of variations in environment associated with the top of the plant on the ascorbic-acid content of the tomato fruit.

METHODS.—Five widely separated locations in the United States having different climatic conditions were selected. These locations included Ithaca, New York, Riverside, California, Berkeley, California, Cheyenne, Wyoming, and Lake Geneva, Wisconsin.⁶ The inbred strain of Bonny Best tomatoes used in experiment II was grown in good garden soil at each location. The soil was undisturbed at Riverside and Lake Geneva but was confined to large containers at all other locations. The plants were grown out-of-doors, except at Berkeley, where the plants were grown in a well-lighted greenhouse. A large sample of soil from three of the locations was shipped to Ithaca, and the same strain of tomatoes was grown in each of the soils. As nearly as possible the same fertilizer and cultural practices were followed for a particular soil sample at Ithaca as was used for the soil at its original location. At the same time at each location the same strain of tomatoes was grown in sand culture, and Hoagland's nutrient solution (4) was used. At all locations the vines were pruned of all axillary growth and supported by stakes. The fruits were picked at each location when completely red and analyzed for ascorbic acid as previously described. Thus two sources of variation on the ascorbic-acid content of tomato fruits were tested: (a) differences between climates when plants are grown in naturally occurring soils and in sand cultures; and (b) effects of different soils in the same environment. The design also permitted a comparison of soil and sand culture as it affected ascorbic-acid content.

RESULTS AND DISCUSSION.—The ascorbic-acid analyses of fruits from plants grown in naturally occurring soils and in sand cultures at the various locations are given in table 3. It is evident that fruit produced on plants grown in naturally occurring soils or in sand culture at various locations varied in ascorbic-acid content. Mean differences well in excess of three times their standard errors are demonstrable, and hence the variation is significant. At Ithaca, Riverside, and Berkeley only slight differences in the ascorbic-acid content of the fruit were observed when soils and sand cultures were compared. In all three locations the mean differences were not of sufficient magnitude to demonstrate P values of 0.01 in a *t* test and hence are not considered as significant.

⁶ The writers gratefully acknowledge the collaboration of Drs. H. E. Hayward, D. R. Hoagland, and A. C. Hildreth.

At Ithaca no special sand culture was set up using Hoagland's solution, since in experiment II a large number of sand cultures were available for comparison with the soil. The figures used in table 3 were those analyses obtained for fruit ripening during the same time interval that fruits were harvested from the soil-culture plots. However, since Hoagland's solution calls for the use of ammonium salts, a separate comparison was later made between solution 25 of experiment II and Hoagland's solution. No significant difference in ascorbic acid was noted between the two treatments, so it is assumed that this comparison is valid.

TABLE 3

ANALYSIS OF ASCORBIC-ACID CONTENT OF TOMATO FRUITS FROM PLANTS GROWN IN SOIL OR IN SAND CULTURE UNDER DIFFERENT ENVIRONMENTS. VALUES EXPRESSED AS MILLIGRAMS OF ASCORBIC ACID PER 100 GM. FRESH FRUIT

LOCATION	PLANTS GROWN			
	IN NATURALLY OCCURRING SOILS		IN SAND CULTURE	
	MEAN \pm SE	NO. OF ANALYSES	MEAN \pm SE	NO. OF ANALYSES
Ithaca, New York.....	24.7 \pm 0.94	17	24.6 \pm 1.13*	30
Riverside, California.....	21.2 \pm 0.65†	23	22.5 \pm 0.92	17
Berkeley, California†.....	18.0 \pm 0.41	14	16.0 \pm 0.75	18
Lake Geneva, Wisconsin...	23.7 \pm 0.67†	15	30.5 \pm 1.12	15
Cheyenne, Wyoming.....	27.1 \pm 1.34	20

* Plants supplied with nutrient solutions of experiment II. Analyses are of fruit which ripened during same time interval as those from soil plot.

† Plants grown in undisturbed soil.

‡ Plants grown in the greenhouse.

At Lake Geneva, however, a significant mean difference of 6.8 ± 1.31 mg. was observed for the ascorbic-acid content of fruits on plants grown in soils and those grown in sand culture. The explanation for this discrepancy is not known, but the following observations may be pertinent. During the early growth of the plants at this location, the sand cultures were watered liberally with nutrient solution, and the plants grew very rapidly (more so than comparable plants in soil). The blossom set of the first blossom clusters was very poor, most of the clusters falling from the plant soon after bloom. The general appearance of the plants indicated an over-abundant supply of nitrogen. In order to assure a good set of fruit, therefore, nutrient solution was withheld from the plants for varying periods of time after first bloom, and the plants were watered with spring water. Nutrient solu-

tion was supplied from time to time to assure adequate growth and good fruit set. These plants grown in nutrient solution at Lake Geneva produced more luxuriant growth than did the plants in sand culture at any other location.

The preceding data indicate that fruit produced on plants growing in a good soil may have the same ascorbic-acid content as fruit produced on plants growing in a complete nutrient solution. The variations which occurred between the two treatments at the one location (Lake Geneva) indicate that the technique of applying nutrient solutions may possibly be important.

The results of ascorbic-acid analyses of fruits from plants grown in different soils, but under the same conditions of climatic environment, are given in table 4. It is evident from the data that the soils used in this experiment do not sig-

TABLE 4
ASCORBIC-ACID CONTENT* OF FRUITS ON PLANTS GROWN
IN DIFFERENT SOILS BUT IN SAME CLIMATIC
ENVIRONMENT AT ITHACA

SOIL FROM	MEAN \pm S.E.	NO. OF ANALYSES
Ithaca, New York.....	24.7* \pm 0.94	17
Berkeley, California.....	24.1 \pm 1.12	16
Cheyenne, Wyoming.....	24.4 \pm 0.94	21
Lake Geneva, Wisconsin.....	26.0 \pm 1.76	11

* Mg. ascorbic acid per 100 gm. fresh weight of fruit.

nificantly alter the ascorbic-acid content of tomato fruits under conditions of the same climatic environment. It is realized that the properties of soils are altered when they are disturbed in transporting from one location to another, and there seems to be no way to correct for this factor. It should be recalled, however, that the soil at three of the locations was placed in large containers, and the handling involved probably represented as much disturbance as was involved in sending comparable soils to Ithaca.

A comparison of the ascorbic-acid values shows much greater differences between fruits produced at different locations than between fruits produced on plants in the different soils at a single location. In fact, all the soils used at Ithaca produced fruit of equal ascorbic-acid content. On the other hand, at Berkeley the average value was 18.0 ± 0.41 mg. per 100 gm., while at Cheyenne the values were 27.1 ± 1.34 mg. 100 gm. Thus the location where the plants are grown may produce a significant influence upon the ascorbic-acid content. While the plants at Berkeley produced fruit relatively low in ascorbic acid, it should be noted that these plants were grown in the greenhouse. Results from experiment IV have a bearing on this point.

In this connection, it should be noted that environment within a given climate significantly affects the ascorbic-acid content of the fruit. From experiment II (table 2) it is evident that significant differences between replications occurred for this character.

When the ascorbic-acid content of fruits produced in selected rows from east to west in the experimental plot was computed, values of 24.6 ± 0.85 , 26.8 ± 1.06 , 27.9 ± 0.93 , and 29.3 ± 1.02 mg. per 100 gm. fresh weight were obtained. Each of these values represents forty or more analyses, and the plants on the east side of the plot produced fruit significantly low in ascorbic-acid content.

EXPERIMENT IV

During the course of the three previous experiments, several additional investigations, relatively simple in design, were carried out. Most of them were conducted concurrently with experiments II and III, and some of the data of the latter are used for comparisons.

At the time the plants were grown out-of-doors at Ithaca in the four different soils, comparable plants were grown in the greenhouse in the same soils. The average ascorbic-acid content of the sixty-five fruits analyzed from the plots outside was 24.7 ± 0.18 mg./100 gm., while the average of fourteen analyses of fruits in the greenhouse was 23.9 ± 1.46 . It is evident from these values that the ascorbic-acid content of fruits produced in the greenhouse is not significantly different from that of fruit produced out-of-doors at the same time of year. The great differences in the acid content of fruits between experiments I and II cannot be ascribed merely to the fact that one was carried out in the greenhouse. Two different sources of seed were used in the two experiments, and it was thought that genetic differences between the plants might explain the variation in ascorbic-acid values. To test this possibility, plants of the same inbred strain as used in experiment II were grown in the greenhouse during the fall and winter of 1941. They were grown in sand culture and supplied with a complete nutrient (treatment 25, fig. 4). Fruits were harvested during late November and early December. The average ascorbic-acid content of twelve fruits was 12.2 ± 0.20 mg. per 100 gm. fresh fruit. These values closely approximate those obtained in experiment I, indicating that the ascorbic-acid content is dependent to a large extent upon the season in which the plants are grown.

Several miscellaneous observations were made during the course of the experiment. At Lake Geneva, besides the two sets of plants grown in sand culture and in soils, a third set of comparable plants were grown in soils but the vines were not staked or pruned. These plants grew luxuriantly and produced the heaviest crop of fruit of any of the three lots. The average ascorbic-acid content of these fruits was 20.5 ± 0.78 mg. per 100 gm. (fifteen analyses). When this value is compared

with a similar value for fruits from supported vines (23.7 ± 0.67 with fifteen analyses), a significant mean difference of 3.2 ± 1.03 ($t=3.11$) is demonstrable. While supporting the vines at this location significantly increased the ascorbic-acid content of the fruit, the magnitude of the differences was not great.

General discussion

These results have indicated that minor variations in the relative concentrations of the macronutrient elements in the nutrient medium may exert a marked influence on the growth and fruitfulness of tomatoes. In general, variations in calcium and nitrate produced more apparent differences over wider ranges of concentrations than were obvious with the other elements. The tallest plants were correlated with low concentrations of calcium, but greatest fruitfulness occurred at calcium levels which were four to six times as high. Similarly, as the concentration of nitrates was increased from 0.0 to 8.5 milliequivalents per liter, growth and fruitfulness were correspondingly greater. While marked variations in plant response could be directly correlated with differences of from 0.0 to 2.8 milliequivalents of the other macronutrient elements, it is evident that some effects were produced by other ranges in concentration. It is difficult, however, to relate these effects directly to variations in the concentration of any individual ion. Thus, at calcium concentrations of 5.7 milliequivalents, much greater fruitfulness resulted when potassium was relatively low and magnesium relatively high. At this same calcium level, however, greatest growth in height occurred when potassium was relatively high and magnesium relatively low. Other examples could be cited which would also illustrate the interaction of ionic effects, even in treatments completely lacking one or more ions.

The results of ascorbic-acid determinations have shown little correlation with variation in macronutrient supply, even though growth and fruitfulness were markedly affected. This may indicate that little variations in ascorbic-acid content would be expected to result from soil differences in so far as such differences are the result of macronutrient supplying power. This is supported by the fact that the four soils tested had no effect. Other soil differences, however, such as physical structure or the ability to supply micronutrients, might be important.

Ascorbic-acid content was found to be correlated with climatic environmental differences associated with season and location. Such differences might include variations in length of day, light quality, light intensity, temperature, etc., and these possibilities are being investigated at the present time.

It seems likely that the effects of climatic environmental factors upon ascorbic acid may have considerable practical importance, especially since many of the fruits consumed during the course of the year are produced in greenhouses during the winter. If, as these results indicate, winter-grown fruits are consistently 50 per

cent lower in ascorbic acid than summer fruit, then the value of tomatoes as an anti-ascorbic agent would be relatively low during the season when the problem of getting an adequate supply of the vitamin in the diet is more difficult. If other varieties behave in a manner similar to this particular strain of Bonny Best and in the winter produce only 12 mg. per 100 gm. of fresh fruit, then the average ascorbic-acid values for tomatoes in various food tables would have to include separate values for summer- and winter-grown fruit. In these experiments winter-grown fruits are lower in ascorbic acid than many canned products which are prepared from summer-grown tomatoes.

Summary

1. Four experiments were carried out to measure the influence of environmental conditions on the ascorbic-acid content of Bonny Best tomatoes. Two of them were designed to test the influence of macronutrient supply and the others measured the influence of climatic environmental factors.

2. In sand cultures, growth and fruitfulness could be correlated with minor variations in nutrient composition.

3. Gross appearances of plants grown in eighty-seven different solutions are discussed and quantitative data presented for growth and fruitfulness. These data are reduced and analyzed by statistical methods.

4. In general, variations in calcium and nitrate in the nutrient medium produced greater differences in growth and fruitfulness of plants over wider ranges of concentrations than were obvious with the other elements. Interactions of the effects of various elements were noted.

5. Ascorbic-acid content of the fruit was significantly higher in some sulphate-deficient treatments and significantly lower in potassium and phosphate deficiencies than average values. With these exceptions, no demonstrable effect of mineral nutrient supply on ascorbic acid was observed.

6. Plants grown during the fall and winter produced fruit with approximately one-half as much ascorbic acid as was produced by comparable plants during the summer.

7. The location where the plants were grown had an effect upon the ascorbic-acid content of the fruit. These effects could not be correlated with differences between soils at the several locations and were apparently associated with differences in the environment of the top of the plant. Significant positional effects on ascorbic-acid content, even in a relatively small trial plot, were observed.

8. Plants grown in sand culture supplied with a balanced nutrient solution produced fruit as high as or higher in ascorbic-acid content than that obtained through the use of a good soil.

9. Several other observations are included relative to the effects of variations

in mineral nutrition on growth and fruitfulness and the influence of environment upon ascorbic-acid content.

10. A discussion of the possible practical significance of the results obtained is included.

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LITERATURE CITED

1. FISHER, R. A., The design of experiments. Oliver and Boyd, London. 1937.
2. HAMNER, C. L., Growth responses of Biloxi soybeans to variations in relative concentrations of phosphate and nitrate in the nutrient solution. BOT. GAZ. 101:637-649. 1940.
3. HAMNER, K. C., and MAYNARD, L. A., Factors influencing the nutritive value of the tomato. Review of the literature. In press.
4. HOAGLAND, D. R., and ARNON, D. I., The water culture method for growing plants without soil. Univ. Calif. Agr. Exp. Sta. Circ. 347. 1938.
5. LYON, C. B., BEESON, K. C., and BARRANTINE, M., Macro-element nutrition of the tomato plant as correlated with fruitfulness and the occurrence of blossom-end rot. Unpublished.
6. MORELL, S. A., Rapid photometric determination of ascorbic acid in plant materials. Ind. and Eng. Chem. (Anal. Ed.) 13:793-794. 1941.
7. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
8. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge University Press, London. 1927.
9. YATES, F., The empire journal of experimental agriculture. 1:129. 1933.

CHROMOSOME FRAGMENTS IN LILIUM WILLMOTTIAE AND HYBRIDS BETWEEN IT AND L. DAVIDII¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 540

J. M. BEAL

(WITH NINE FIGURES)

Chromosome fragments have been reported in *Lilium japonicum* (2, 3, 4, 5), *L. henryi* (2, 3, 4), and *L. formosanum* and *L. tigrinum* (4). *L. willmottiae* can now be added to the list, since more than 95 per cent of the microsporocytes in smear preparations from plants of this species growing at Wychwood, Lake Geneva, Wisconsin, have shown one fragment in addition to the twelve normal bivalents.

The fragment is easily detectable at diakinesis and at late metaphases of meiosis I (fig. 1), since it generally passes intact to one pole of the spindle before the bivalents disjoin. At late anaphase of meiosis I (fig. 2) it lies near the attachment constriction region of the anaphasic group of chromosomes and later is included with the chromosomes of this dyad nucleus when the nuclear membrane forms.

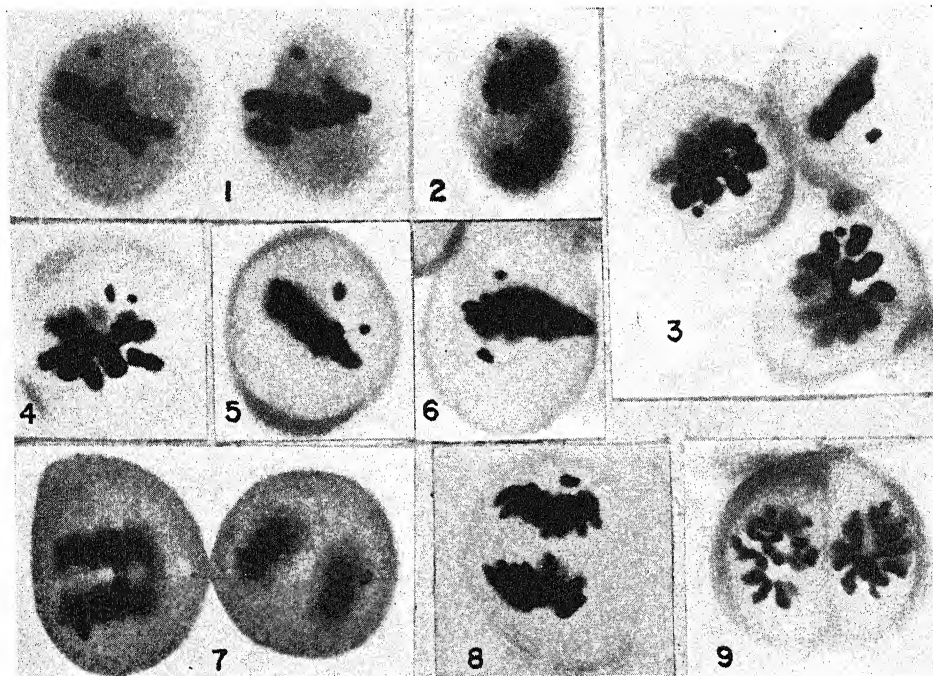
The behavior of the fragment during meiosis II has not been determined. The fragment is short and compact and its structure obscure. In several instances it has appeared as a compact double body as it passes toward the pole in meiosis I; hence it is possible that it may divide in meiosis II and thus provide a fragment for two of the four quartet nuclei and microspores. It may not divide in meiosis II, however, and in this event only one of the four microspores would possess the fragment in addition to the haploid number of twelve chromosomes.

The point of immediate interest associated with the fragment is that it is found in certain hybrids between *L. willmottiae* × *L. davidii* and the reciprocal cross. Microsporocytes from a single slide of *L. davidii* have failed to show fragments in this species during diakinesis and metaphase I. This is not decisive proof of their absence, but evidence from the hybrids to be mentioned shortly indicates the nonoccurrence of fragments in this species.

During the summer of 1937, crosses were made between *L. willmottiae* × *L. davidii* and *L. davidii* × *L. willmottiae*. Viable seeds were obtained as a result of both crosses and numerous seedlings have been established. All the seedlings resemble *L. willmottiae* much more closely than the other parent, and the hybrids are larger than either parent. Many of them produced flowers in the summer of

¹ This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

1940 and again in 1941. Anthers from a number of the flower buds were smeared, and the microsporocytes fixed in Navashin's solution and stained according to the gentian violet-iodine method, but no record was kept of the individual plants from which the various slides were made. Twelve of these slides show either first or second meiotic divisions, six being from the *L. willmottiae* × *L. davidii* cross and six from the reciprocal. Of the twelve slides, one shows no fragments in any of the



FIGS. 1-9.—Fig. 1, *L. willmottiae*, metaphases I. Fig. 2, same, late anaphase I. Fig. 3, *L. willmottiae* × *L. davidii*, metaphase I. Figs. 4-6, same, metaphase I, two fragments in each sporocyte. Figs. 7, 8, *L. davidii* × *L. willmottiae*, anaphases I. Fig. 9, same, metaphase II. Fragment in left dyad at right of upper center.

sporocytes. This slide was made from one of the plants resulting from the *L. davidii* × *L. willmottiae* cross, which indicates that fertilization had been accomplished by a gamete from a pollen grain which did not possess a fragment. One slide from the *L. willmottiae* × *L. davidii* cross showed two fragments (figs. 4-6) of unequal sizes, while the remaining ten slides representing both crosses all show one fragment in practically every sporocyte (figs. 3, 7-9).

The origin of the second fragment is unknown and it is smaller than the one which regularly occurs in *L. willmottiae*. Both fragments (fig. 5) may pass to the same pole at metaphase I, or they may go one to each pole (fig. 6). Their behavior

in meiosis II has not been determined. In all the hybrids which contain a single fragment in the sporocytes the fragment behaves as described for *L. willmottiae*.

The occurrence in *L. willmottiae* of the fragment which is passed, apparently unchanged, to hybrid progeny—whether *L. willmottiae* is used as the seed or the pollen parent—may perhaps be unexpected and raises several questions which cannot at present be answered. For example, does the fragment possess an attachment constriction? Its regular passage to one pole of the spindle and its subsequent inclusion in one of the dyad nuclei suggest that it may, but its compactness at diakinesis and metaphase I makes it extremely difficult to determine. In numerous instances the fragment has appeared to be double at anaphase I, thus suggesting that it may divide at meiosis II. It is possible, however, that no spindle attachment is present and that the fragments may behave in the same manner as X-ray-induced fragments in the neuroblasts of *Chortophaga viridifasciata*, reported by CARLSON (1). These fragments, lacking spindle attachments, show behavior and distribution similar to the chromosomes, indicating that polar repulsions effect the equatorial position at metaphase; that the separation of the chromatids is autonomous; that the orientation and migration toward the poles is due either to protoplasmic streaming or to an expanding "Stemmkörper"; and that the attachment constriction functions mainly in making the orientation and separation of the chromosomes uniform and equal. Whether the observations of CARLSON are applicable to the *Lilium* species and hybrids reported here will have to await the outcome of more detailed studies now under way.

The fragments have shown no evidence of being reduplications of sections of the normal chromosomes, such as MATHER (3) reported for *L. japonicum* and *L. henryi*, since in none of the sporocytes examined has there been any indication of pairing between the fragment and a major chromosome.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. CARLSON, J. G., Meiotic behavior of induced chromosomal fragments lacking spindle attachments in the neuroblasts of the grasshopper. *Proc. Nat. Acad. Sci.* 24:500-507. 1938.
2. MATHER, K., The chromosomes of *Lilium*. II. *R.H.S. Lily Yearbook* 3:38-40. 1934.
3. ———, Meiosis in *Lilium*. *Cytologia* 6:354-380. 1935.
4. SANSOME, E. R., and LA COUR, L., The chromosomes of *Lilium*. III. *R.H.S. Lily Yearbook* 3:40-45. 1934.
5. SATO, M., Chromosome studies in *Lilium*. I. *Bot. Mag. Tokyo* 46:66-88. 1932.

TOXIC EFFECTS OF SODIUM PENTACHLORPHENATE AND OTHER CHEMICALS ON WATER HYACINTH

A. A. HIRSCH

In many streams of southern United States profuse growths of water hyacinth (*Crassipes eichornia*) impede navigation, retard drainage flows, favor mosquito breeding, and prevent fishing. Eradication has been confined to treatment with hazardous arsenicals or to mechanical processes, such as removal by raking or the recent maceration development.

The experiments described here were the preliminaries in a search for a safe poison to control this growth in earth-lined drainage canals near an urban area. Of particular interest for trial were two recently introduced materials: sodium pentachlorophenate, an industrial algicide and fungicide marketed as Santobrite by the Monsanto Chemical Company of St. Louis, Missouri, and Beneclor 3, a chlorinated hydrocarbon, manufactured by the Chloroben Corporation, Jersey City, New Jersey. Copper sulphate was also included for observation. A control set completed the system.

Seventy-eight mature plants were collected from a nearby bayou and divided equally into four 14-gallon, painted, iron washtubs stored outdoors and almost filled with water. River silt was placed in the bottoms of the tubs. Santobrite and copper sulphate were added every 2 weeks from a stock solution to give increments varying from 5 to 20 p.p.m.; beneclor 3, a liquid, was pipetted directly in amounts equivalent to 5-10 gallons per acre. The toxins were thoroughly mixed with the water in the tubs. The amount of additional dosage was decided from the physical appearance of the plants. This incremental treatment was adopted in order to establish the approximate lethal limits employing the minimum number of experimental units. The experiment terminated in advance of cold weather to avoid killing by the first freeze. The results are summarized in table 1.

Transfer of plants from their natural habitat to the tubs caused some stunting; nevertheless all plants remained healthy, unless poisoned, and produced flowers at various times. Dwarfing was especially noted at low initial doses of santobrite, but specimens treated with low dosages of copper sulphate and beneclor 3 seemed to thrive even better than the controls. When concentrations of 45 p.p.m. copper sulphate and 45 gallons per acre of beneclor 3 were reached, these sets were abandoned because the toxicity was insufficient to warrant further observation. While some damage was inflicted by beneclor 3, the lethal dose on 2 weeks' con-

tact was probably much higher. Even the initial dose of santobrite caused yellowing of stalks and withering of leaves; further applications damaged tissues more definitely and dwarfed new leaves. About 35 p.p.m. seemed to arrest growth, but complete necrosis was obtainable only after reaching a concentration of 80 p.p.m. This strength is about 2.7 times the maximum recommended for control of algae

TABLE 1
SUMMARY OF RESULTS

DOSAGE	CONTROL	COPPER SULPHATE	BENECLO 3	SANTOBRITE
Smallest necessary to affect appearance.....		35 p.p.m.	35 gal./acre	5 p.p.m.
Necessary for complete kill.....		Not reached	Not reached	80 p.p.m.
Maximum reached.....		45 p.p.m. (abandoned)	45 gal./acre (abandoned)	80 p.p.m.
Final appearance of survivors.....	Good condition; few brown spots	Like control except more widespread browning (all original plants living)	Two nearly dead; others extensively necrotic	All dead

in industrial cooling systems and is too low to cause skin irritation, even on prolonged contact.

The physiological action of santobrite suggests two field procedures for combating the problem of the water hyacinth: (1) A light application, somewhat more than 5 p.p.m., for retarding growth in areas where complete removal is not essential or in zones where other aquatic life is to be preserved. (2) Complete elimination by a dosage approximating 80 p.p.m. This method is applicable especially to reach into shallow or inaccessible tributaries in connection with a maceration unit.

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EFFECT OF HEAT-DRYING UPON THE PERIDERM OF WASHED POTATOES¹

RICHARD O. BELKENGREN AND EDWIN S. CIESLAK

For some years it has been the practice of commercial potato growers to wash the tubers prior to shipment. This removes the adhering soil as well as permitting a more thorough rejection of those obviously defective or rotten. The usual procedure has been to convey the potatoes through a pre-sizer, then to a washing tank or spray chamber, and finally to a picking table discharging into sacks or crates. Loading wet or damp potatoes has resulted in considerable rot in transit, and drying equipment has been installed at some packing houses. As a rule, the drying immediately follows the washing and precedes the picking.

In the operation of one particular drying installation (1), it was noticed that the potatoes when shipped were extremely resistant to spoilage, both in transit and subsequent storage. When more than 1000 carloads of potatoes subjected to this treatment resulted in no spoilage claims whatever, it was concluded that the protection afforded could not be accounted for solely on the basis of external moisture removal. The drying procedure subjects the potato to a moderately high velocity movement of hot air in the course of passage through the unit. That the effect was not due entirely to removal of inoculating organisms was established by experiments in which the tubers were artificially inoculated externally with various organisms which might cause spoilage. With other conditions remaining constant, the treated potatoes continued to show greater resistance to rot than did the controls, indicating the probability of a biological change within the tuber itself.

The potato periderm consists of three parts: the phellem, several layers of heavily suberized cells very much flattened; the phellogen; and the phelloderm, several layers of cells lying next to the cortical parenchyma. The cell walls of these layers protect the inner portions. In freshly harvested potatoes, only a few layers of cells are present, but with time the number of cell layers increases. This increase is affected by a number of factors, chief of which are temperature and moisture.

Two varieties, a white potato and a red one, Bliss Triumph, were used in the experiments. Samples were obtained from commercial driers and from laboratory-dried tubers prepared under similar conditions. Controls consisted of samples taken immediately before and immediately after treatment. Other samples were taken 3-6 days after treatment, in order to show the change.

¹ This work was done under the sponsorship of F. C. Peters, grower, Goulds, Florida.

Pieces of tissue 6 mm. square by 3 mm. deep were cut from the surface of the tubers. These were killed and fixed in Allen-Bouin fluid for 3-6 hours, washed in 50 per cent alcohol, dehydrated in 70, 95, and 100 per cent alcohol, cleared in xylene, slowly infiltrated with paraffin, and finally imbedded in a paraffin block. From each piece of tissue, thirty random 18- μ sections were taken from the middle one hundred, fixed on slides, freed of paraffin, hydrated to 50 per cent alcohol, stained in safranin, washed and destained if necessary, counterstained with Delafield's haematoxylin, dehydrated in successive changes of alcohol, cleared in xylene, and mounted in balsam. Four random sections out of each thirty were measured.

The averages of 1440 measurements and cell counts are given in table 1. Fifteen potatoes were used in each group. These samples were given the indicated heat treatment in a laboratory drying oven.

TABLE 1

RESULTS OF HEAT-DRYING ON POTATO PERIDERM, MEASURED 72 HOURS AFTER TREATMENT

GROUP	TREATMENT	PHELLEM		PHELLOGEN-PELLODERM		TOTAL PERIDERM (μ)	TOTAL NO. OF CELLS
		THICKNESS (μ)	NO. OF CELLS	THICKNESS (μ)	NO. OF CELLS		
A.....	Control	77.82	5.63	63.23	3.93	141.05	9.56
B.....	63° C. for 8 minutes	82.99	6.18	73.72	4.21	156.71	10.39
C.....	78.5° C. for 4 minutes	94.24	6.23	106.70	4.77	200.94	11.00

Group B averaged 0.83 cell layers and group C 1.44 cell layers greater than the control (group A). The 4-minute exposure to the higher temperature favored a greater increase in thickness in 72 hours than the longer exposure at the lower temperature. The cell walls in groups B and C were also thicker than those of the controls. This is attributed to increased suberization, probably promoted indirectly by the higher temperature, since storage of potatoes at low temperatures retards suberin formation. Control lots of potatoes dried without heating showed no increase in cells after 72 hours. Immediately following the drying period, the periderm showed a slight decrease in thickness, presumably due to drying.

Recently harvested Bliss Triumph potatoes, treated on a commercial scale by the same heat-drying process, showed an increase in periderm cell layers from 8.7 to 10.9. Results on samples obtained from another similar commercial unit and shipped to this laboratory showed 11.53 cell layers in the periderm of heat-dried potatoes against 10.47 for the controls, an increase of 1.06 layers. Thickening of the phellem cell walls was also noted.

The increase in periderm thickness is especially noted in about 72 hours and appears to be initiated by the drying treatment. The increase and the suberization are marked in young potato tubers having a relatively thin periderm at the start. Obviously the heat which the tuber absorbs in the drier subsequent to removal of its external moisture acts as a stimulant for the formation of periderm. Possibly the change is hormonal in character. Apparently the effect is due, at least in part, to this heat rather than to mere drying alone. In support of this, THORNTON (2) reports that periderm formation normally does not take place so rapidly under dry conditions as in a moist environment. Additional work in progress in this laboratory is now aimed at a fuller understanding of the effect.

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LITERATURE CITED

1. RUEHLE, G. D., Bacterial soft rot of potatoes in southern Florida. Univ. Florida Agr. Exp. Sta. Bull. 348. 1940.
2. THORNTON, NORWOOD, Oxygen regulates the dormancy of the potato. Contr. Boyce Thompson Inst. 10:339. 1939.

CHROMOSOME NUMBER OF ZANTHOXYLUM AMERICANUM¹

RUTH I. WALKER

(WITH EIGHT FIGURES)

The haploid chromosome number of 35 was reported for staminate plants of *Zanthoxylum piperitum* by SINOTÔ (2). He observed that the chromosomes of pollen mother cells at metaphase of the first meiotic division are similar in size, except for a few which are larger. One of these larger chromosomes appears to be a univalent and is always found at the periphery of the equatorial plate. He frequently saw, however, an unequal pair of chromosomes. NAKAJIMA (1), working with staminate plants of the same species, also observed that the reduced number of chromosomes is 35. He found an unequal pair of chromosomes and a small univalent.

Because of these reported differences, and since no account has appeared concerning the chromosomes of the American species *Zanthoxylum americanum* Mill., the study reported in the present paper was made.

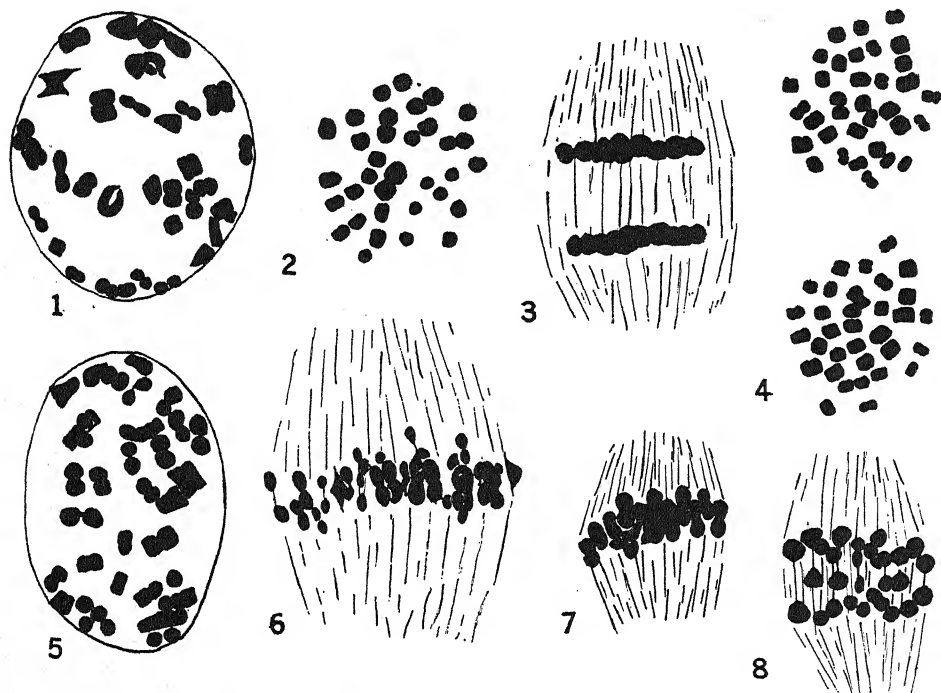
Staminate and pistillate buds were collected from plants of *Z. americanum* near Sauk City, Wisconsin, and in Milwaukee, Wisconsin. The material was fixed in Flemming's medium and Carnoy's acetic-alcohol-chloroform solutions. The fixed material was imbedded in paraffin, cut at 8-12 μ , and stained with Heidenhain's iron-alum haematoxylin or crystal violet.

A study of the pollen mother cells of plants from each locality at diakinesis and at the equatorial plate of the first meiotic division showed 34 pairs of chromosomes (figs. 1, 2). At metaphase these are ovoid and are similar in appearance, except for size. During the anaphases the movement of the chromosomes toward the poles is normal (fig. 3), although occasionally the chromosomes of a pair may lag or reach the poles earlier. At metaphase of the second meiotic division 34 chromosomes are present on each spindle (fig. 4). Fewer preparations were obtained of the macrospore mother cell during meiosis, but in such figures as were found 34 pairs of chromosomes are present (fig. 5).

Studies were also made of lateral views of microspore and macrospore mother cells during first metaphases and anaphases to determine the form and number of the chromosomes. Many preparations were examined, but owing to the size,

¹ This research was done with the assistance of W. H. Falvey of Work Projects Administration, University Natural Science Project, Work Project no. 10324.

number, and crowding of the chromosomes, it was impossible to distinguish all pairs of chromosomes distinctly in one view. Of the chromosomes observed, however, all are paired and the chromosomes of each pair are of the same size and form (figs. 6-8). At no time is there any indication of a univalent or of an unequal



FIGS. 1-8.—*Zanthoxylum americanum*: Fig. 1, pollen mother cells at diakinesis. Fig. 2, polar view of equatorial plate; first meiotic division. Fig. 3, anaphase. Fig. 4, polar views of equatorial plates; second meiotic division. Fig. 5, macrospore mother cell at diakinesis. Fig. 6, pollen mother cell, lateral view of equatorial plate; first meiotic division. Fig. 7, macrospore mother cell, lateral view of equatorial plate; first meiotic division. Fig. 8, anaphase. All approximately $\times 2800$.

pair of chromosomes in *Zanthoxylum americanum*, as was reported by SINOTÔ (2) and NAKAJIMA (1) for *Z. piperitum*.

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LITERATURE CITED

1. NAKAJIMA, G., Cytological studies in some dioecious plants. *Cytologia Fujii Jub.* Vol. Pp. 282-293. 1937.
2. SINOTÔ, Y., Chromosome studies in some dioecious plants. *Cytologia* 1:109-191. 1929.

BRIEFER ARTICLE

MACERATION METHOD TO DEMONSTRATE THE VASCULAR SYSTEM IN ZEA MAYS

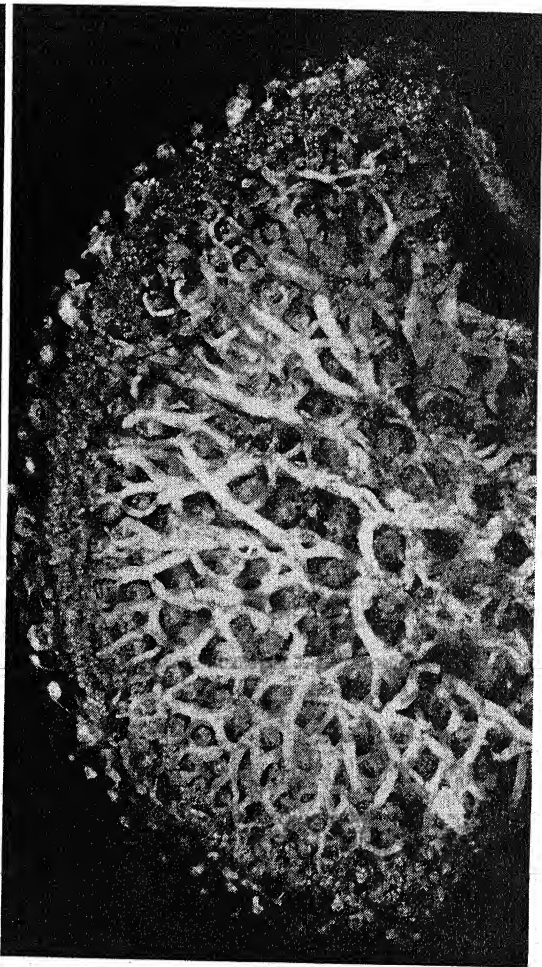
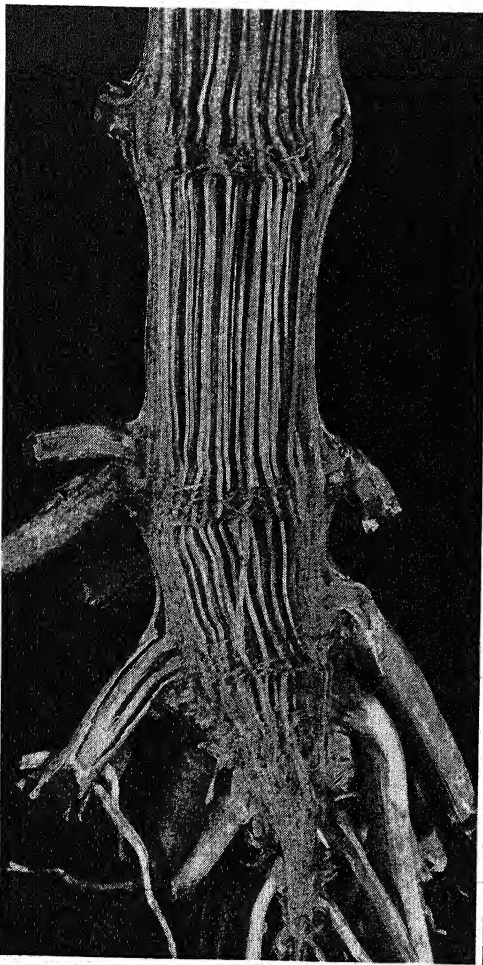
(WITH TWO FIGURES)

The maize axis has long been used as a monocotyledonous stem type, and generations of students have examined it in transverse section. When the question of the course of the bundles is raised, however, few can give even a general description, especially of the behavior at the node, since it is well nigh impossible to elucidate the construction by serial sections. A note by EVANS¹ caused the writer to work out the following method whereby abundant class material of completely separated vascular skeletons may be simply and permanently prepared (fig. 1). The same method could probably be applied to other material; the writer has even used it successfully on sugar-cane stem which had been preserved in formalin-acetic-alcohol.

The method relies on the fact that it is possible to remove the parenchyma and ground tissues by rotting and yet leave intact the vascular bundles and highly lignified parts. Mature maize plants, after careful removal from the soil, have the leaves and roots trimmed to within about $\frac{1}{4}$ inch of the axis, care being taken to leave the epicotyl and the first few internodes. The stem is now washed completely free of soil and then split into two, preferably in the plane of the leaves. The splitting is best done by starting at the epicotyl with a scalpel, and when the wider parts of the axis are reached, changing to a stronger knife or even to a chisel and mallet if the specimen is large and well matured. Some of the upper part of the stem is now removed, since for class use a whole axis is unwieldy and there is no need to include more than one or two nodes above the region from which arise the highest roots. The specimen should be trimmed to its final size and shape at this stage, as it is much more difficult to do this on the finished skeleton. If, when the axis is shortened, the cut is made above the node, the nodal anastomosis will be clearly displayed in the final preparation (fig. 2).

The prepared halves are now placed in any convenient receptacle which is not too deep, covered with tap water, and allowed to decompose. Rotting of the ground tissue is hastened if the water is kept warm (about 70° F.). The addition of ten to twenty crushed peas or maize grains also seems to hasten the process, probably by insuring rapid initial increase in the number of bacteria. Additional water is necessary from time to time to replace that lost by evaporation. The material must always be completely covered, since if any part remains above the liquid, dark stains—which are impossible to remove—result from the growth of molds just above the liquid surface. After a few weeks, the time depending largely on the temperature of the water, the parenchyma

¹ EVANS, A. T., Vascularization of the node in *Zea mays*. BOT. GAZ. 85:97-103. 1928.



FIGS. 1, 2.—Fig. 1 (left), base of maize stem split longitudinally and then "retted." Fig. 2 (right), network of anastomosing bundles at node.

will have rotted and the center of the stem feel soft and gelatinous. A strong jet of water will now free the vascular bundles from the ground parenchyma. The washing is conveniently done by using a jet from a piece of rubber tubing, which allows wide control of the volume and rate of flow of the water. After the center has been freed from debris and a strong jet forced through the nodal regions in all directions, a jet is put on the exterior, cleaning up the outside and usually removing the internodal epidermis in large sheets. The outer bundles do not fall apart, as they are all imbedded in the subperipheral sclerenchyma band. If the rotting is not complete, the stem is cleaned up as much as possible and then returned to the tank for another week or so.

The skeleton is best kept as a dry specimen. It should be placed in 90 per cent alcohol for a day or two and then allowed to dry at room temperature, a process which takes about 12 hours. The alcohol serves to remove the unpleasant smell which the specimen has when first taken out of the tank and also to enable the specimen to be dried without much shrinkage or collapse.

When finished, the skeleton, which should be free from odor and almost white in color, is strong, durable, and well able to stand up to the handling received in class use.—
B. C. SHARMAN, *Botany Department, The University, Leeds 2, England.*

CURRENT LITERATURE

Compuestas Bonaerenses: Revisión de las Compuestas de la Provincia de Buenos Aires, la Capital Federal y la Isla Martín García. By ANGEL L. CABRERA. Extracted from the Revista del Museo de La Plata (Nueva Serie) Tomo IV, Sección Botánica. 1941. Pp. 450. Figs. 145. Pls. 10.

A study is presented "of all the species of Compositae found in the Province of Buenos Aires, in the Federal District (City of Buenos Aires) and in the Island of Martín García which lies in the Río de La Plata." In the entire country of Argentina the Compositae, with about 200 genera and about 1200 species, constitute approximately one-sixth of the phanerogamic flora. In the restricted area studied, 102 genera, containing in all 299 species, are found to occur. Of these, "74 genera and 245 species are native. The rest are adventitious or adapted from cultivation."

The author presents full and carefully drawn descriptions, together with lists of specimens studied and often with critical observations. The numerous figures are mostly full-page, carefully executed etchings, many of them by the author himself. These should prove of great value, especially in the case of the less well-known species. The ten plates are given over to twenty habit-photographs of selected species. Keys are given to the various tribes as well as to the component genera and species. The Astereae, Inuleae, and Heliantheae are seen to be the best represented tribes, while the genera with the most species are *Eupatorium* (19), *Baccharis* (29), and *Senecio* (28). Several new species and varieties are described and there are a few new combinations. A bibliography of 118 citations (pp. 433-437) and a concise summary in English (pp. 438-439) are appended. The book as a whole represents a noteworthy contribution to the literature on Compositae.—E. E. SHERFF.

The Structure of Protoplasm. Edited by WILLIAM SEIFRIZ. Ames, Iowa: Iowa State College Press, 1942. Pp. vii+283.

One of the most important summaries of protoplasmic structure in the English language, this monograph is the first to be published by the American Society of Plant Physiologists. It contains the papers presented before the organization at Philadelphia in December, 1940. The original symposium was arranged by Dr. SEIFRIZ, and the volume is edited by him. Much of the excellence of arrangement and the integration of the symposium depended upon his keen analysis of the field and his choice of men to handle each subject. Following the introduction, written by SEIFRIZ, these topics are discussed: Microscopic structure of the cell wall, by C. W. HOCK; Proteins and protoplasmic structure, by L. W. MOYER; Molecular structure in protoplasm, by O. L. SPONSLER and JEAN D. BATH; Some mechanical properties of sols and gels and their relation to protoplasmic structure, by HERBERT FREUNDLICH; Structural differentiation of cytoplasm, by G. W. SCARTH; Structural differentiation of the nucleus, by C. L. HUSKINS; Protoplasmic streaming in relation to gel structure in the cytoplasm, by D. A. MARSLAND; Relation of the viscosity changes of protoplasm to amoeboid locomotion and cell division, by W. H. LEWIS; Physical aspects of protoplasmic streaming, by NOBURÔ KAMIYA; and Some physical properties of protoplasm and their bearing on structure, by WILLIAM SEIFRIZ. A short supplement contains a brief communication on protein and protoplasmic structure by K. H. MEYER, of Geneva, and a letter from W. T. ASTBURY, of Leeds, England.

The paper by FREUNDLICH was the last thing he wrote before his death. It will be prized by his admirers as will also be the fine portrait which appears as a memorial to him.

The material is well illustrated, and the individual papers carry literature lists which add much to the usefulness of the volume. The press work has been beautifully done under the watchful eyes of Dr. W. E. LOOMIS, of Iowa State College, who provides the foreword.

Its up-to-date points of view, and the documentary evidences of structure presented, make this monograph an indispensable work for all students and investigators of protoplasmic phenomena.—C. A. SHULL.

Advances in Colloid Science. Edited by E. O. KRAMER and collaborators. New York: Interscience Publishers, Inc., 1942. Pp. xii+434. \$5.50.

The editors have planned a series of volumes on the advances of colloid science, to appear whenever conditions warrant publication. This first volume of the series contains a dozen monographic reviews with the following titles and authors: The measurement of the surface areas of finely divided porous solids by low temperature adsorption isotherms, by P. H. EMMETT; The permeability method for determining specific surface of fibers and powders, by R. R. SULLIVAN and K. L. HERTEL; A new method of adsorption analysis and some of its applications, by ARNE TISELIUS; Solubilization and other factors in detergent action, by J. W. MCBAIN; Recent developments in starch chemistry, by K. H. MEYER; Frictional and thermo-dynamic properties of large molecules, by R. E. POWELL and HENRY EYRING; The constitution of inorganic gels, by H. B. WEISER and W. O. MILLIGAN; The creaming of rubber latex, by G. E. VAN GILS and G. M. KRAAY; Streaming birefringence and its relation to particle size and shape, by J. T. EDSALL; Synthetic-resin ion exchangers, by R. T. MYERS; The study of colloids with the electron-microscope, by T. P. ANDERSON; and Anomalies in surface tensions of solutions, by R. A. HAUSER.

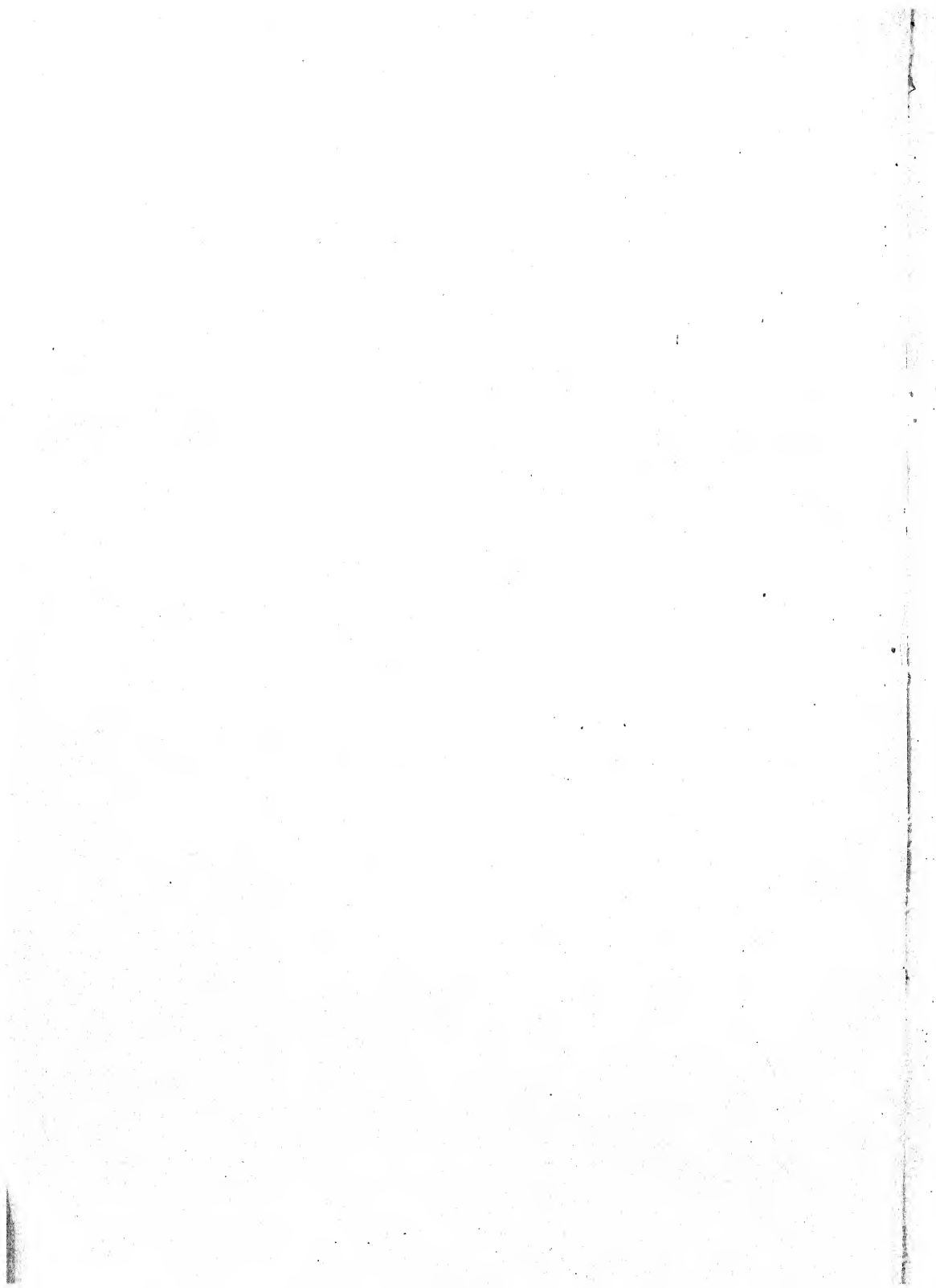
Each monograph carries its own bibliography, in some cases short lists but in others over 100 references. They are written in clear style and will prove exceedingly helpful to anyone interested in these rapidly progressing fields of investigation and application of science to practical problems. The editors deserve congratulations for the initiation of this series of reviews, a number of which come from European laboratories. Many of the reviews contain material of special significance to physiological investigations.—C. A. SHULL.

Desert Wild Flowers. By EDMUND C. JAEGER. Stanford University Press, 1941. Pp. 30+322. Illustrated. \$3.50.

First published in 1940, this book has now been revised by the inclusion of an artificial but workable key to the 764 species of California desert plants which are illustrated by line drawings (a few by photographs). It should now have an even wider appeal to all nature lovers in this area, who may identify easily and rather positively most of the plants which they are likely to encounter. No grasses are included.—C. E. OLMSTED.

Soils and Fertilizers. By FIRMAN E. BEAR. New York: John Wiley and Sons, Inc., 1942. Pp. xiii+374.

The third edition of this book, formerly entitled *Soil Management*, which has just appeared, has been revised and improved in various ways. Although some of the headings have been changed slightly, the twenty-six chapters follow the same order and treat the same topics as in earlier editions. It is commended especially to beginners in the study of soils and fertilizers, for it is simply and concisely written and deals with the principles without confusing them with too much detail. It is a very good elementary text in its field.—C. A. SHULL.



THE BOTANICAL GAZETTE

June 1942

THE FOOT OF *RIELLA AMERICANA* AND ITS RELATION TO NUTRITION OF THE SPOROPHYTE

R. A. STUDHALTER

(WITH SIXTEEN FIGURES)

Introduction

Of the three parts of the sporophytic generation in the bryophytes, the foot and seta have received much less attention than the sporangium. Once almost universally considered to function both as an anchoring and as a necessary absorbing organ, the foot has recently been re-examined, both as to structure and function, particularly in connection with the frequent partial nutritional independence of the sporophyte from the gametophyte. This paper presents an account of its developmental stages, confirms evidence of sporophytic independence, and adds some anatomical considerations which suggest a possible reversal of the usually accepted parasitic relations between the two generations.

The fullest anatomical accounts of the foot in liverworts are found in papers by LEITGEB (19) and MEYER (22, 23, 24), with summaries by GOEBEL (13). For the moss foot, the most complete papers are those of LORCH (20, 21) and BLAIKLEY (2). For the several species of the aquatic liverwort *Riella*, the foot has received scarcely more than a mention from LEITGEB (19), PORSILD (26), and CAVERS (9), and developmental stages have apparently not been studied. HOWE and UNDERWOOD (15), describing *R. americana*, do not mention the foot, and none of the illustrations shows details of its tissues. Up to the present there has been no description of the mature foot and no account of the ontogeny of this organ for the species just mentioned.

In nearly all liverworts and mosses the foot originates by division of cells at the base of a more or less elongated seta. Its shape at maturity varies, but it is gen-

erally a smoothly rounded or oval structure imbedded more or less deeply in the gametophytic tissues at the base of the calyptra.

Deviations from such a smoothly rounded pattern have been known since 1874, when KIENITZ-GERLÖFF (16) showed that the peripheral cells of the foot of *Frullania* protrude slightly into the gametophytic tissue. LEITGEB (19) found a similar papillate condition in *Frullania*, *Aneura*, *Mörkia*, *Riella helicophylla*, and *R. parisii*; in *R. helicophylla* some of the cells are elongated and extend from middle to periphery of the foot. Much longer protruding processes were discovered (19) in the foot of *Anthoceros* and *Dendroceros*. Still longer penetration filaments were reported by GOEBEL (11) for the moss *Diphyscium*, and later (12) for *Eriopus*, where they originate on the seta instead of on the foot. Superficial foot cells vary in the different genera from smooth or slightly elevated to very long, branched filamentous structures. Many nouns and descriptive adjectives have been applied to these extensions, including papilla, papillate, papillose, haustorium, haustorial outgrowth, root hair, haustorial rhizoid, haustorial tube, rhizoid-like tubule, outgrowth, tubular outgrowth, process, protrusion, appendage, and filament. As a substitute for most of these terms, the following are proposed in the hope that they will confer a greater relative value. Peripheral cells which have the superficial contour of the foot are called smooth. The term gibbous is applied to those which are rounded at the margin of the foot. For longer penetrating structures the terms subdigitate and digitate are proposed, and for structures long enough to resemble rhizoids or root hairs, the term filament. Throughout, the noun process or protrusion is suggested for the protruding cell or portion of a cell, when this is longer than that represented by the term gibbous. Common descriptive adjectives are also needed, as forked, branched, and irregularly branched.

In addition to the papers already cited, gibbous cells or processes of various degrees of extension have been described or illustrated in the foot by PORSILD (26) for *Riella paulsenii*, LANG (18) for *Notothylas*, O'KEEFE (25) for *Targionia*, LORCH (21) for mosses as *Bryum*, BLAIKLEY (2) for a number of mosses and *Anthoceros*, and VRABER (28) for *Riella helicophylla*. Often these were merely simple observations, or even only diagrams without mention in the text. The most common interpretation, when any was given, has been that the processes serve as absorbing organs (13). BARTLETT (1), however, considered that many of the protrusions attributed to the foot in *Anthoceros* belong in reality to the gametophyte. BUCH (6) accepted the similarity, both in structure and function, of the processes in the foot of the Anthocerotales to the rhizoid of the gametophyte. BLAIKLEY noted that, in general, those mosses in which the contact between the two generations is the closest are the most likely to have their peripheral foot cells extended into processes. BOWER (5) observed that the processes of the foot increase its absorbing surface and extend its range of absorption.

Enlarged cells in the foot have been found by CAMPBELL (7) in *Geothallus*. Enlarged nuclei are described in the same genus (7), as well as in *Eriopus*, by GOEBEL (12), in *Plagiochasma* by MEYER (23), and in the mosses by BLAIKLEY (2). Abundant, or a dense, deeply staining protoplasm was seen in *Geothallus* by CAMPBELL (7), in *Eriopus* by GOEBEL (12), in *Corsinia* and *Plagiochasma* by MEYER (22, 23), in *Targionia* by O'KEEFE (25), and in mosses by BLAIKLEY (2). Thickened or otherwise modified cell walls were reported by GYÖRFFY (14) for *Molendoa*, and by LORCH (20) and BLAIKLEY (2) for various mosses. Modifications in the gametophytic cells adjacent to the foot were noted in *Corsinia* by MEYER (22), in *Targionia* by O'KEEFE (25), in *Anthoceros* by CAMPBELL (8), and in several mosses by BLAIKLEY (2).

That the sporophyte in both liverworts and mosses has a completely dependent nutritional existence, except in the Anthocerotales, is still rather commonly accepted, as seen in quotations cited by BOLD (3). CAMPBELL (7), in fact, interpreted abundant cytoplasm and large nuclei as an indication that the foot cells were actively engaged in the nutrition of the sporophyte of *Geothallus*. GOEBEL (12) considered that the processes of the seta of *Eriopus* transferred food materials from both foot and vaginula into the seta and thence into the sporangium. As early as 1908, however, DOVIN (10) noted the presence of chloroplasts in all cells of the foot of *Sphaerocarpus*, the closest relative of *Riella*. MEYER (23) believed in the heterotrophic nutrition of the young sporophyte of *Plagiochasma*, but in at least partial nutritional independence in later stages, since chloroplasts and glycose were found in foot and sporangium. The same writer (24) found the foot cells and sporangium wall cells of *Corsinia* to have a green color. In 1924, CAMPBELL (8) kept an excised sporophyte of *Anthoceros* alive for 3 months and discussed its nutritional independence. GOEBEL (13) recognized the probable independence of the sporophyte of some of the bryophytes. BOWER (5) stated that the sporophyte of *Buxbaumia*, which also possesses processes in the foot cells, becomes nutritionally independent in its more mature stages. STUDHALTER (27) demonstrated complete nutritional independence during the latter half of the development of the sporophyte of *Riella americana* and *Sphaerocarpus texana* by growing excised sporangia to maturity in tap-water cultures; these not only reached their full term, but they ripened their spores, which in turn germinated normally and abundantly. BOLD (3, 4) found chloroplasts and included starch in the foot, seta, and capsule of various liverworts and mosses; at least partial nutritional independence was indicated for the sporophyte.

Material and methods

The plants upon which this study is based were collected in the Davis Mountains of Texas or else grown in laboratory cultures. Fixing was done in formalin-

acetic-alcohol, and staining with safranin or Delafield's haematoxylin or with various combinations. The usual paraffin method was used. Both stained and unstained whole mounts were studied for comparison.

Observations

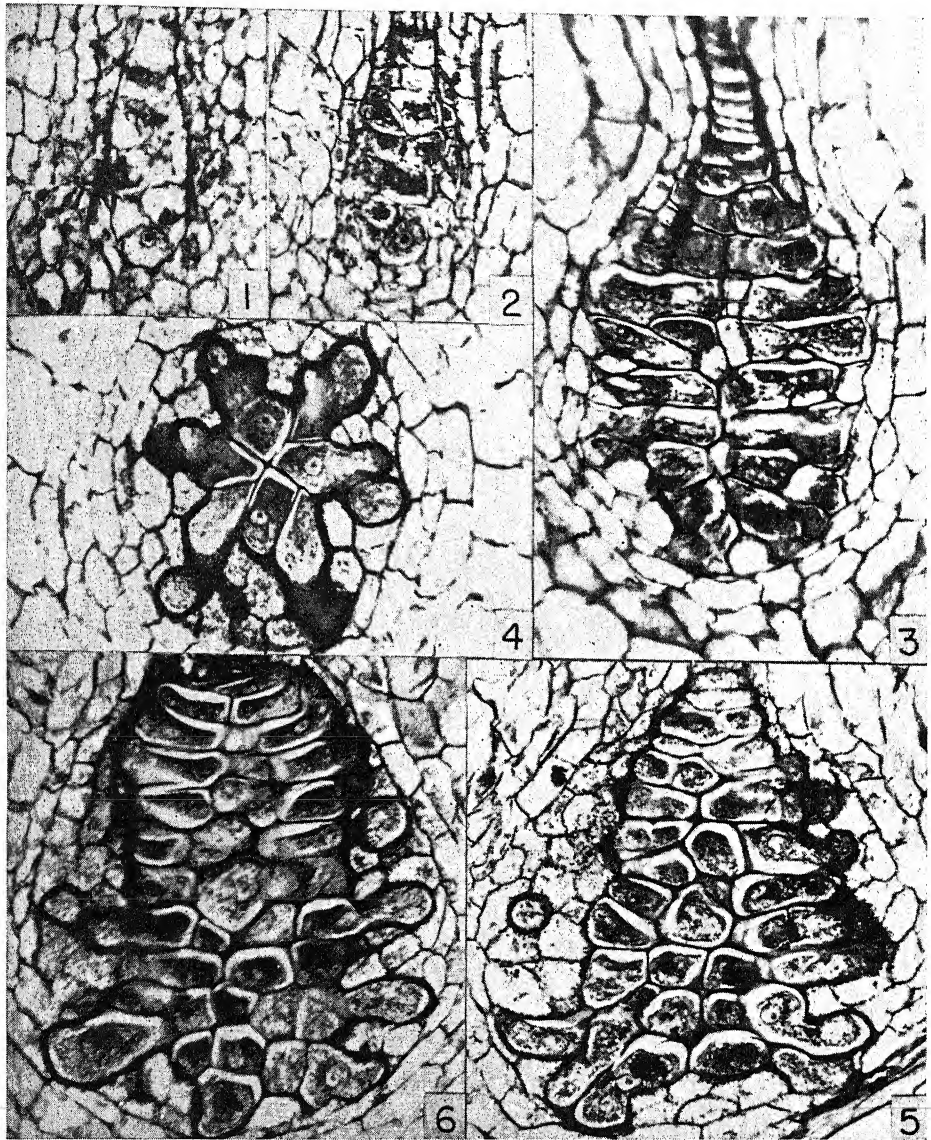
ORIGIN AND EARLY GROWTH OF THE FOOT

In the eight-celled stage, the sporophyte is divided by oblique walls in such a manner that a single cell points downward into the tissues of the subcalyptral pad. From this cell soon originates a row of four to eight short cylindrical cells which, by the upward growth of the gametophytic tissues, come to be deeply imbedded in the latter. The walls of these cells are uniformly thin, and the cross walls are perpendicular to the axis; cell divisions have taken place in a single plane.

The basal portion of this young seta broadens slightly, and further cell divisions in the basal region ensue in new planes, resulting in several oblique cross walls (fig. 1). This stage, the first definitely to be recognizable as a foot, makes its appearance when the sporange has a diameter of seven or eight cells, of which the outermost layer has been differentiated into a relatively thick sporange wall. The cells of the very young foot are isodiametric, angular toward the interior of the foot, rounded at its periphery, and of the same size as most of the cells of the subcalyptral pad. Their cell walls are uniformly thin, not darkened, and stain much as do the walls of the cells of the calyptra and involucre. The nucleus is large and prominent and usually occupies a central position; it is oval or spherical, and has a diameter range of $8-13\ \mu$, with an average of about $7.5 \times 10\ \mu$. There is a single spherical nucleolus which measures about $5\ \mu$ in diameter. It is surrounded by a clear zone extending out to the thin but prominent nuclear membrane. These sporophytic nuclei remain the same size throughout the development of the foot and are much larger than those of the calyptra and involucre, which average $5-7\ \mu$ in diameter. The cytoplasm is meager in amount and occupies a peripheral position. When the nucleus is centrally located, a thin layer of cytoplasm surrounds it and several thin cytoplasmic strands connect with the peripheral cytoplasm. In early stages, therefore, there is a large, clear central vacuole.

The zone of contact between the foot and the calyptra is nearly always intimate, there being no evidence either of mucilage or of intercellular spaces. The latter are also lacking within the foot.

The first oblique and irregular divisions are soon followed by more regular ones, involving vertical walls near the median line of the foot (fig. 2). As a result, the cells at this stage tend to lie at the same level in pairs, and a pair extends completely through the center of the foot from one margin to the opposite one. The median cross walls are not all in the same plane. In a young foot five cells long and definitely only two cells wide, the sequence from top to bottom (in which wall 1 at



FIGS. 1-6.—Fig. 1, oblique longisection of young foot showing its differentiation from lower end of seta. Fig. 2, longisection just off median line showing increase in mass of cytoplasm, beginning of plasmolysis, and binucleate cell. Fig. 3, longisection of foot with gibbous to subdigitate, binucleate, radially elongated cells; cytoplasm becoming coarsely granular. Fig. 4, cross-section through middle of foot with digitate, radially arranged giant cells, walls of variable thickness, and discontinuous overlapping wall near center. Fig. 5, longisection of foot with gibbous and digitate cells, one of latter (at left) bent at right angles and cut in cross-section. Fig. 6, longisection of foot shown in fig. 7, just off median line, showing thickened walls, discontinuous and overlapping at two points in upper portion. $\times 270$.

the top of the foot is used as a basis for orientation in a circle) was as follows: wall 2, 30° to the right of wall 1; wall 3, 60° to the right of wall 1; wall 4, 30° to the right of the first wall; and wall 5, again 60° to the right of wall 1. At any given level, four cells often appear to radiate from the center, 60° - 90° apart. Occasionally there appear to be six cells around the central point, instead of four.

There is naturally a tendency for a cell to be narrow at the middle of the foot and to broaden tangentially toward the periphery. Many cells are broadly triangular in cross-section of the foot. The margin of the foot remains for a time essentially smooth, and the entire foot has the shape of a much elongated oval. Cells measure $20\text{--}25 \times 25\text{--}40 \mu$ at this stage, while the entire foot measures $30\text{--}50 \times 40\text{--}80 \mu$. Plasmolysis is sometimes pronounced.

MATURING FOOT

Profound changes in the structure of the foot usually occur about the time this organ is eight to ten cells long (fig. 3). Simultaneously, the seta has a length of ten to twelve cells, and the sporange, which has not yet differentiated spore mother cells and nurse cells, has a diameter of about ten cells.

Growth in size of the foot proceeds steadily, until at maturity a length of nine to twelve cells or $200\text{--}250 \mu$ or more is reached and a width of $150\text{--}200 \mu$. In shape the foot is broadly ovate. Figure 7 shows the relations between sporange, seta, foot, calyptra, involucre, and subcalyptral pad in a sporophyte in which nurse cells and spore mother cells had become differentiated.

Perhaps the most obvious changes in the maturing foot are those associated with the shape of the constituent cells. At first broadly triangular in outline, they gradually change into many different shapes. With increase in the diameter of the foot, the cells elongate radially (fig. 3). Remaining narrow at the center, they usually broaden materially toward the margin, especially as seen in cross-section (fig. 4). Since the cells are so compacted that intercellular spaces are virtually absent, some of them grow in various directions and attain many grotesque shapes, in both cross- and longisection. Occasionally long, very slender cells are found extending from center to margin (figs. 5, 14). At the base the cells abandon their truly radial direction of growth and extend downward in lines more nearly parallel with the axis of the foot (figs. 3, 5). Many cells, even at later stages, extend from the median line of the foot outward to its margin (figs. 9, 10, 12, 13). Cell diameters vary greatly, no matter in which direction a section has been cut. Often with increasing age cross walls are formed which divide the radially elongated cells into two or three shorter ones. The fundamental plan of cells extending radially is found in all parts of the foot.

The arrangement of four cells radiating from the center about 90° apart is often

in evidence (fig. 4), even toward maturity of the foot. More frequently, however, the plan is actually or apparently obliterated, so that four to seven cells appear to radiate from near a common point.

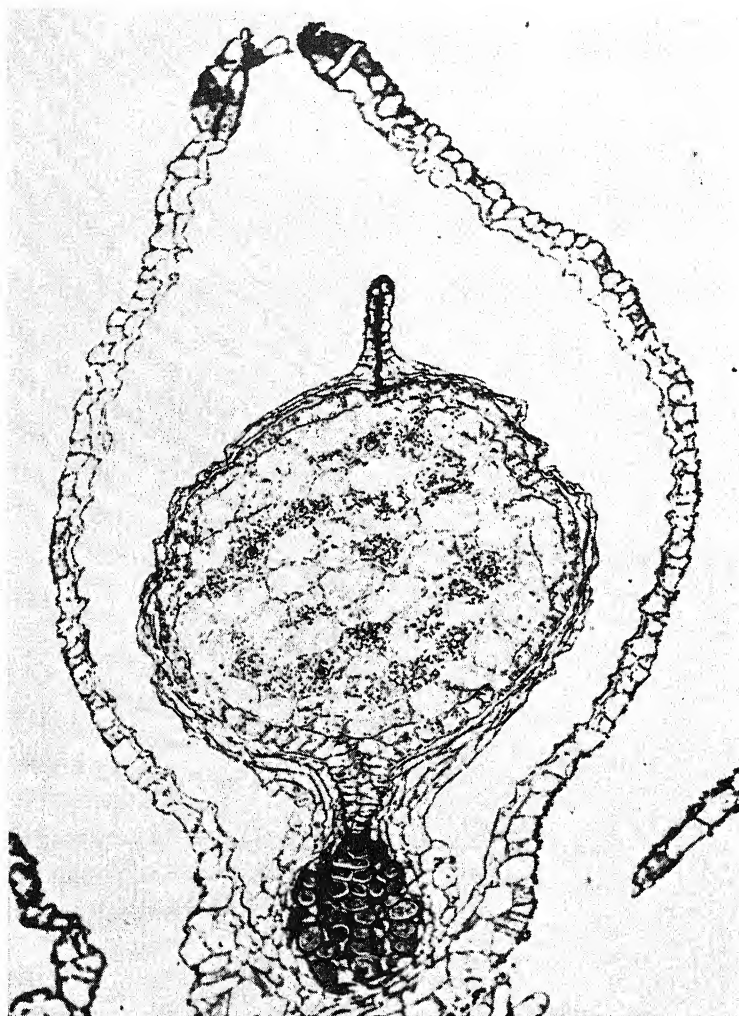


FIG. 7.—Longisection through involucre and its ostiole, calyptra, archegonial neck, sporangium, seta, and foot, the latter cut slightly to one side of median plane. $\times 67$.

It is at the margin of the foot that the cells assume their most grotesque shapes. Many become bent, twisted, or deeply lobed or forked into equal or unequal parts, with the free tips varying from angular to very broadly rounded (figs. 9, 10, 12, 13). Individual cells frequently become gibbous and remain so to their maturity

(fig. 5). Others extend farther out as subdigitate processes, which penetrate more or less deeply among the cells of the subcalyptral pad (fig. 3). Still others are elongated into truly digitate processes, which protrude deeply into the gametophyte (figs. 4, 5, 6, 12, 14). The latter may be straight, twisted, or sharply bent, sometimes even at right angles; in a longisection of a foot it sometimes happens that some of them are cut lengthwise, while others, which had grown at right angles, are cut in perfect cross-section (fig. 5, left). A longisection or an oblique one through the margin of the foot often cuts through no other part of this organ than the digitate processes, each of which has the appearance of a circular, thick-walled cell (fig. 8). In cross-section such a process may be $42\ \mu$ in diameter. Digitate protrusions are more numerous in the lower half or two-thirds of the foot. All types of processes classified in the introduction as having been described in certain other liverworts and mosses have been found in *Riella americana*, with the exception of filaments.

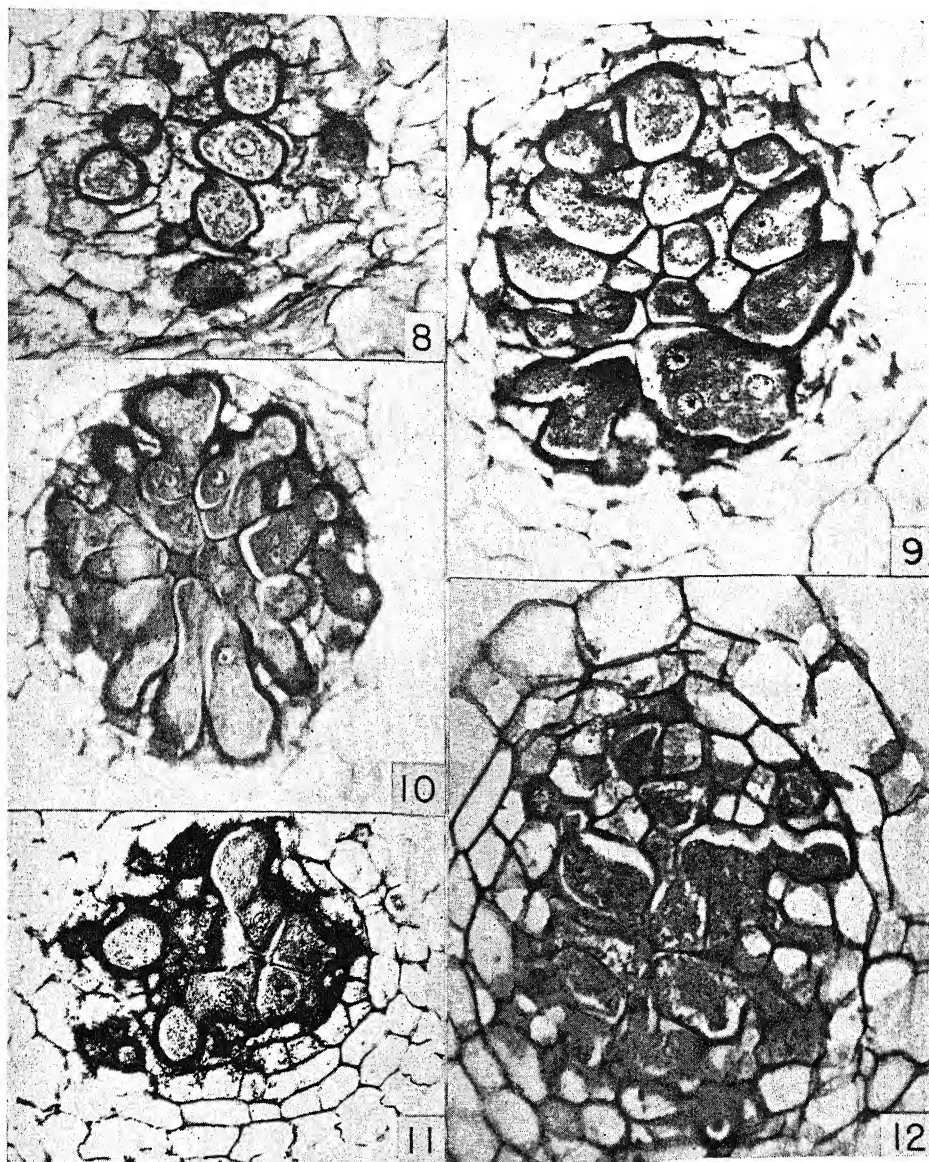
The actual three-dimensional shape of the foot cells ranges from the one extreme of rather simple boxlike structures to the other of irregularity which defies description. In general, however, the longest dimension lies radially in the foot, and the cells are broader in cross- than in longisection of this organ.

There is also great variation in the size of the cells. Some of the smaller ones are little larger than those of the surrounding gametophyte (fig. 9). A large proportion of them are giants, however, not only in length but often in breadth as well (figs. 4, 9, 10, 11, 12, 13). Since they often extend half way through the foot, it is not surprising to find them measuring $100\ \mu$ in length; a few reach at least $110\ \mu$. Some of the wider unbranched ones measure up to $70\ \mu$ in width. A long narrow cell was found to measure $20 \times 100\ \mu$, as seen in one section. Lobed and forked cells may be as wide as $70\ \mu$.

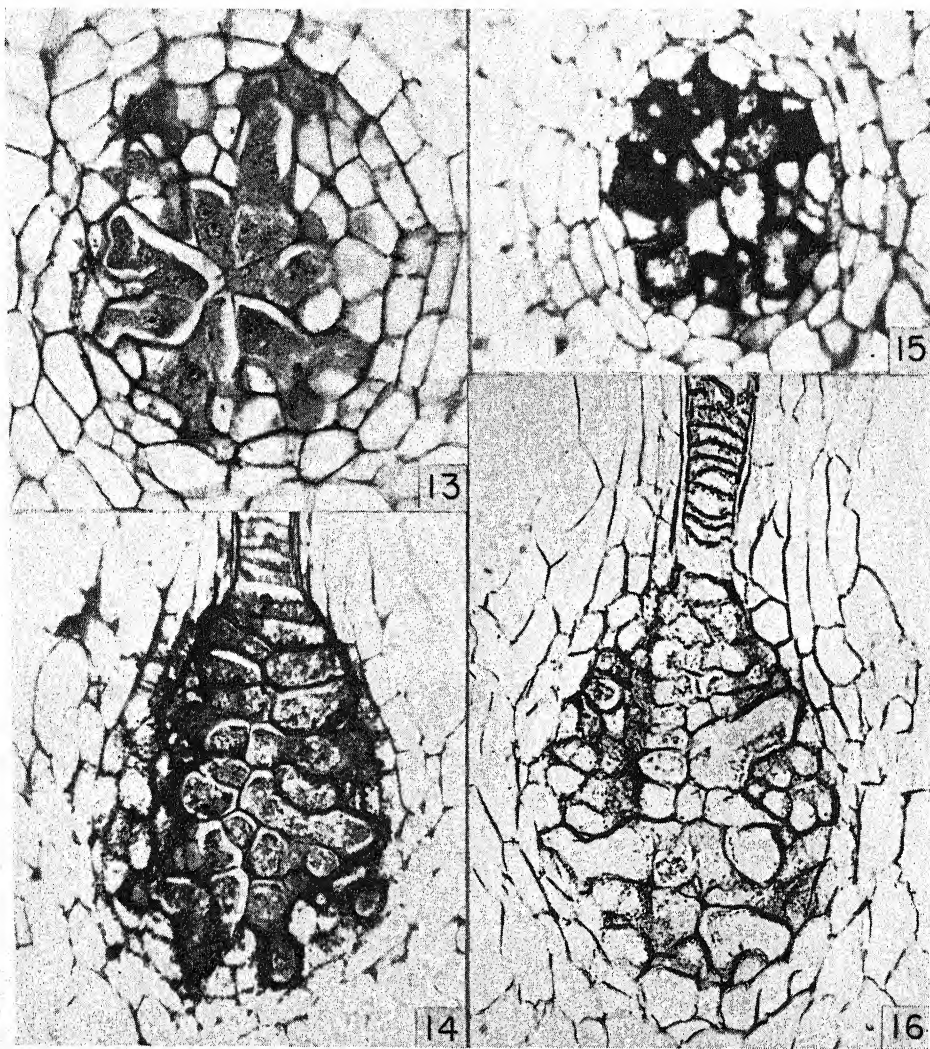
It is difficult to estimate the total number of cells in the mature foot. At maturity this organ is ten to twelve cells long. Assuming that the majority of cells extend from middle to periphery, there should be forty to sixty cells in all. But since some of them are further divided radially, the total number is probably much greater.

With such variation in cell shape, it is not surprising that the margin of the foot is usually very irregular. Although the general shape is oval, the actual zone of contact between foot and gametophyte is usually a very irregular line. This boundary is generally a definite one (figs. 4, 7); at other times it is not clearly defined, since those cells of the latter which are adjacent to the sporophyte (one to three rows) occasionally become somewhat modified (fig. 14). The contact between foot and gametophyte, however, is almost without exception intimate throughout its development, lacking both mucilage and intercellular spaces.

The first change to take place in the cell wall is one of thickening and darkening.



FIGS. 8-12.—Fig. 8, longsection of extreme edge of foot shown in fig. 7, consisting of cross-section through several digitate processes, one showing nucleus. Fig. 9, oblique longsection through side of foot showing forked cell, giant cell with four nuclei (nucleoli of two of them present in adjacent section), and long irregular cell just above the latter. Fig. 10, cross-section through middle of foot with digitate, forked, and irregular giant cells, cleaved cytoplasm in lower forked cell, and much variation in wall thickness. Fig. 11, oblique section through upper part of foot of small sporophyte approaching maturity, with variable wall thicknesses. Fig. 12, cross-section below middle of foot with giant branched cell at middle right which shows two parallel partial walls, at free end of one of which lie two nuclei at different optical levels (only one shows in photograph). $\times 270$.



FIGS. 13-16.—Fig. 13, cross-section farther below middle of same foot as in fig. 12, showing granular degeneration of nuclei and cytoplasm, and partial wall in cell at left. Fig. 14, longisection, almost median, of mature foot showing granular degeneration of protoplasm of foot cells and some thick-walled granular cells in adjacent gametophyte. Fig. 15, cross-section just below middle of foot of dwarf mature sporophyte showing thick walls and degenerated protoplasm. Fig. 16, longisection of very old foot in last stages of granular degeneration, with varied walls. $\times 270$.

The former change, which at first proceeds at a uniform rate over the entire cell, soon becomes irregular and results in much variation in thickness in different parts of the foot (figs. 4, 10, 13), in a single cell (figs. 10, lower; 13, left), or even in a single short wall (fig. 13, lower middle). Extreme cases of variation in different parts of the foot are shown in figures 11 and 15. Although one of these is a cross-section and the other oblique, it is probable that all the thickest walls shown have been cut obliquely; they measure 4-5 μ in width, which may be interpreted as 3-4 μ in actuality. Cell walls appear commonly thicker at the periphery than in the interior of the foot; this is due only in part to a larger percentage of oblique cuts through the rounded marginal portions of the cells. A great deal of variation is also found in a single wall, which may taper rapidly from very thick to quite thin within a short length.

The color of the wall varies from nearly colorless in the younger foot, progressively through deeper shades, to a very dark black. It is never yellow, which is the color assumed by the mature seta. Staining does not appear to have an appreciable effect on the shade of color exhibited in the darker walls.

In the young foot all walls are typically continuous, although some of them may be folded, as in the crotch of a lobed or forked cell. Frequently in older cells the wall is interrupted; it may have the appearance of having been broken, with one or both ends displaced laterally (figs. 5, 16). At other times the ends have become altered so that they do not appear any longer to fit together. The probable cause of such interruption is often a simple breaking at a weak point in the wall; often, however, a portion of the wall seems to have been absorbed. At other times the walls are both discontinuous and overlapping, with each end frequently tapering to a long point (figs. 4, 6). Partial walls are also found (figs. 12, right; 13, left). These extend at various angles toward the interior of a cell, sometimes only for a short distance, at others nearly to the opposite wall. Such a partial wall may be uniform in thickness, with a blunt or rounded end, or it may taper to a point. In rare instances a partial wall extends more deeply into the cell at one optical level than at another, as shown by its passing directly over the top of a nucleus (fig. 12, right). There is usually no discernible definite point on the opposite wall at which the partial wall had once connected with the former. It seems most likely that the partial wall is the result of partial absorption by the protoplasm. Perhaps some of the other wall irregularities are due to the same factor. Such absorption, of course, results in the coalescence of adjacent cells; but it should be emphasized that partial walls are not very common, that most walls are continuous, and that nearly all the giant cells have attained their size by enlargement.

Long before the foot is mature or the cells have reached their maximum size, a limited free nuclear division takes place. Two nuclei per cell (figs. 10, 13) are common, often so common that half the cells of a single section are binucleate. Three

nuclei are less common and four even less so. At times a single cell will show three or four nuclei in the same section (fig. 9). There is no definite proof of more than four nuclei in one cell. Binucleate cells are first found in a relatively young foot (fig. 2). The formation of binucleate cells by the dissolution of a cross wall and the fusion of the respective protoplasts is possible, but this occurs only at infrequent intervals. Likewise, the fusion of two binucleate cells would theoretically give rise to a cell with four nuclei. Since three or four nuclei are found in giant cells which lack partial walls, apparently free nuclear division is the more common cause of the multinucleate condition. Nuclei are the same size in uninucleate and multinucleate cells. The position of a nucleus is usually central; in a digitate process it is always located some distance back from the tip.

Just as nuclear division began in the relatively young foot, so also are several changes in the cytoplasm first seen at a very early stage in the development of this anchoring organ. The first noticeable change is a great increase in the mass of the cytoplasm (figs. 2, 3, 4), which soon occupies virtually the entire space within the cell wall. The large central vacuole becomes obliterated, to be replaced by numerous small irregular clear spaces in all parts of the cytoplasm. Shortly afterward these also disappear. With increasing age, the cytoplasm becomes progressively coarser in structure, the granules increasing materially in size (figs. 3 *ff.*). A single cell is not necessarily uniform in the type or degree of granulation (fig. 9, lower right). The color of the cytoplasm varies from its original colorless condition through darker shades to a deep black. Frequently different portions of the cytoplasm of a single cell vary appreciably in color and density; extremes of light gray and jet black may appear side by side in a single cell. Very dark cytoplasm is especially prominent in highly plasmolyzed cells.

Plasmolysis of the cytoplasm is common and begins early (fig. 2). In an older foot the cytoplasm may be drawn completely away from the wall, so that it occupies a central position. The shrinkage is not due to fixing or staining agents but to factors within the plant itself. The shrunken masses are often irregular in shape and at times angular.

Occasionally the cytoplasm is cleaved into two or several parts (fig. 10) without the formation of a cross wall. The parts may differ in color and granulation; they may be widely separated in the cell, lie in close proximity, or be superimposed.

COMPARISON OF FOOT CELLS WITH GAMETOPHYTIC TISSUES

In nearly every respect, the cells of the foot differ from those of the calyptra, involucre, and subcalyptral pad. These differences revolve around the shape, size, and arrangement of the cells; the thickness, color, composition, and continuity of the walls; the size, prominence, and number of nuclei; the granulation, color, cleavage, and plasmolysis of the cytoplasm; and finally the absence of vacuoles.

However, the cells of the gametophyte in the one to three rows adjacent to the foot may become somewhat modified (figs. 13, 14). This is particularly noticeable in the slight thickening and darkening of their walls and in the slightly increased granulation of their cytoplasm. They neither increase in size nor become multinucleate, nor are the other changes described for the cells of the foot visible in them. Nevertheless, such modifications as are present sometimes make it difficult to be certain of the exact boundary line between foot and gametophyte. Again, nearly all the morphological aspects discussed for the foot of *Riella americana* appear to be absent from the foot of almost every other genus of liverwort or moss; apparently they are absent also from the foot of other species of *Riella*.¹

DEGENERATION OF FOOT

The time at which degeneration begins in the foot is difficult to determine. Some phases, at least, appear long before the foot reaches its mature size. This is true of the coarse granulation of the cytoplasm, of severe plasmolysis, and of the partial breakdown of certain walls. Most of the cells affected, however, continue to increase in size and reach their maturity before final decomposition sets in. Occasionally growth of the entire foot as well as of the sporangium is retarded, presumably by an earlier or a more severe onset of degeneration, so that the mature foot is a dwarf with cells which are smaller than usual (figs. 11, 15). Once the foot has reached its mature size, no further changes take place in its general outline (fig. 16). The alterations accompanying degeneration are mostly protoplasmic.

Discontinuous, overlapping, and partial walls, already discussed, are a part of cell degeneration. Much of this breakdown has taken place before the foot has reached its maximum size. Nearly all the walls remain intact and rigid, and the cells retain their shape to the end.

There is little or no change in the size of the nucleus. The first noticeable modification in this structure is a cloudiness and granulation of the perinucleolar zone (cf. figs. 4 and 13). The nucleolus, which in its prime stains brilliantly, becomes muddy black in color. In later stages it and the surrounding granules disappear, leaving an apparently empty nuclear membrane; or the membrane and granules may disappear first, leaving a discolored nucleolus. Finally all traces of the nucleus vanish (fig. 16), often before the cytoplasm has completely degenerated.

Granulation of the cytoplasm continues uninterruptedly, sometimes accompanied by cytoplasmic cleavage. There may be a massing of the granules, which occurs in any part of the cell, frequently against the cell wall. The masses of granules gradually become smaller and less dense and ultimately disappear completely, leaving a lumen empty of all solid materials (fig. 16). True vacuoles may

¹ Uninucleate subdigitate, digitate, and branched cells are illustrated in the foot of *Riella affinis* by THOMPSON (Amer. Jour. Bot. 29: 278. 1942).

be said to have disappeared with severe plasmolysis and clumping of the cytoplasm. Such open spaces as appear in the degenerating cytoplasm can hardly be called vacuoles. To the very end, the contact between foot and gametophyte is a very intimate one, with little or no evidence of intercellular spaces and none whatever of mucilage (fig. 16).

The final result of these degeneration phases is a foot composed of empty cell walls which, however, remain rigid (fig. 16). Degeneration is virtually complete by the time the sporange is mature and sometimes before the spores are ripe. At this time the seta may be eighteen to twenty-three cells long and a yellowish brown color.

With ripening of the sporange, it breaks off at the upper end of the seta, leaving this organ and the foot imbedded in the subcalyptral pad. Sometimes the seta, which has been yellow and apparently nonfunctional since early stages, breaks off later at its lower end. Calyptra and involucre break off at their bases. Ultimately, if the gametophytic plant continues growing (which is usually the case), seta, foot, and subcalyptral pad are shed, followed by a smoothing over of the axis of the plant at this point.

Discussion

The anomalous structures described in this paper for the foot of *Riella americana* have been found, with only minor variations of degree, to be present in every foot examined. They may be interpreted along two major lines, in both of which the outstanding consideration is the established nutritional independence of the sporophyte of this species during the latter half of its development.

FUNCTION OF FOOT. —Present ideas of the function of the foot have been much modified from the older theory of anchorage in the gametophyte and absorption of water and elaborated food materials from that generation. Since *R. americana* is an aquatic plant, it constitutes an ideal object for the study of the nutrition of the sporophyte. In simple tap-water culture, lacking added minerals or nutrients, it has been found (27) that the immature as well as the mature excised sporange can absorb all the necessary water and mineral substances directly from the surrounding medium through its wall cells. It is only a step further to the absorption of these materials through an added thin calyptra, which is in direct contact with the water of the habitat. And since the sporophyte is well able to manufacture all its needed food materials, as shown in simple water culture, it seems clear that the foot, at least during the latter half of its development, does not function as an absorbing organ; or that if some absorption does take place from the gametophyte, this is purely incidental. Very little starch is present in the foot. The seta, however, gives a positive starch test before its maturity. The sporange is well supplied with chloroplasts and starch. It is probable that the developing sporange absorbs

starch from the calyptra, with which it is in intimate contact; but this also is incidental, as shown by the water cultures.

The facts that degeneration phenomena in the cells of the foot have their beginning rather early in its ontogeny, and that they are often complete before maturity of the sporophyte, lend corroborative evidence. In this species, at least, the digitate processes cannot be considered as necessarily increasing the absorbing surface, or as extending the zone from which absorption can take place, unless they represent vestigial structures from a former age during which the sporophyte was less independent from a nutritional point of view. Some other explanation would seem at least as likely.

With the elimination of the absorptive function, only the function of anchorage remains. This function it certainly performs efficiently, since the sporangium is held firmly in place, even when the plant is growing on a rock in swiftly running water, which it occasionally does. Anchorage, however, is aided indirectly by both calyptra and involucre. Again, the deeply penetrating digital processes aid in anchoring, even though this also can scarcely be considered their major significance. In any event, it seems that the foot of this aquatic species now has only one major function, that of anchorage.

POSSIBLE REVERSAL OF PARASITIC RELATIONS.—Nutritional independence on the part of a sporophyte can be attained without the anomalous modifications of the foot described in this paper. In the genera studied by BOLD (3, 4) the foot is apparently usually simple and unmodified, as in most of the liverworts and mosses. It seems at least possible that the significance of these modifications may lie in a reversal of the more usually accepted parasitic relations between the two generations.

There is much literature dealing with the pathological anatomy of the cells of an infected host, both plant and animal. No effort is made here to review these papers, since the general plan is well described for host plants by KÜSTER (17). Affected host cells commonly undergo such modifications as enlargement to giant size; becoming multinucleate; increase in size of the nuclei; increase in mass and granulation of the cytoplasm; cleavage of the cytoplasm without formation of cross walls; thickening, resorption, and other modifications of the cell wall; and granular or other degeneration of nucleus and cytoplasm. Such conditions are summarized by KÜSTER for wound tissue, for tissues with gummosis, and for galls induced by such varied organisms as bacteria, fungi, hemiptera, mites, and nematodes. The actual presence of the parasite is not needed to induce such changes. WATKINS and WATKINS (29), for example, show that cotton roots merely placed in contact with the mycelium of the cotton-root rot organism, or treated with extracts of infected host tissues, give similar results. It should be stressed again

that such anatomical modifications occur in the tissues of the host plant and not in those of the parasite.

The anatomical changes described in this paper for the cells of the foot of *R. americana* include phenomena very similar to those of plant host cells infected or infested with a parasite: giant, multinucleate cells; enlargement, dissolution, and other modifications of the cell walls; increase in the mass of cytoplasm; and granular decomposition of the cytoplasm and nucleus. The formation of digitate processes can well be considered a part of the formation of giant cells.

But these modified cells are in the foot, which, according to the older accepted idea, is a part of the parasitic generation. From KÜSTER's summary of the relation of host and parasite, they should be in the host generation, namely, in the gametophyte, according to the older theory. It would appear, then, that the foot in this case represents the host and not the parasite. This evidence is tentatively interpreted as indicating a reversal of the usual parasitic relationship between the generations.

It is probable, nevertheless, that the young sporophyte is for a short time partially dependent upon the gametophyte for a portion of its elaborated food supply. This is borne out by the failure of cultured sporanges to make much growth when excised during an early stage in their ontogeny (27). As the sporophyte grows older, however, it soon becomes completely independent, nutritionally, from the gametophyte. And the anatomical evidence here presented would seem to indicate that still later the parasitic relationship may possibly become reversed, that possibly the gametophyte exerts somewhat of a parasitic action on the sporophyte. The problem is complicated by the fact that one or two rows of cells in the subcalyptral pad lying adjacent to the foot also show a few slight modifications, especially a small amount of thickening and darkening of the cell walls. This phenomenon may at times be associated with the intimate contact between sporophyte and gametophyte and the occasional difficulty in deciding whether a given cell near the boundary line belongs to the one or the other. Often, however, cells definitely belonging to the gametophyte show a slight modification. But, in general, all the foot cells are modified as though they were affected host cells, while the cells of the surrounding gametophyte remain unmodified, or essentially so.

The hypothesis of reversed parasitism is proposed only tentatively and is based thus far only on anatomical evidence.

Summary

1. The foot of the aquatic liverwort, *Riella americana*, arises from a broadening of the base of the young seta in a manner comparable with the early ontogeny of the foot of other liverworts.

2. A series of profound anatomical modifications begins when the foot is eight to ten cells long and proceeds uninterruptedly to its maturity and final degeneration. Most of the cells of the foot become enlarged into giant cells, which at its margin become gibbous and later commonly send digitate processes into the tissues of the surrounding gametophyte. The cell walls become thick and dark, the interior ones being sometimes partly resorbed so as to leave partial or overlapping walls. The nuclei are uniformly large; there are usually two and sometimes three or four in each cell. The cytoplasm becomes plasmolyzed and coarsely granular. Both cytoplasm and nucleus exhibit granular degeneration at or before the time of ripening of the spores.

3. The zone of contact between foot and gametophytic tissues is a very intimate one.

4. Since nutritional independence had been previously demonstrated for this species, the foot is considered as having, at least at maturity, the single major function of anchorage and none of absorption from the gametophyte.

5. Anatomical evidence, such as the formation of multinucleate giant cells and granular degeneration of the protoplasm, suggests the possibility of a reversal of the usually accepted nutritional relation between the two generations; the gametophyte of this species may prove to be parasitic upon the sporophyte, except in its early ontogeny.

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LITERATURE CITED

1. BARTLETT, EMILY M., A comparative study of the development of the sporophyte in the Anthocerotaceae, with special reference to the genus *Anthoceros*. Ann. Bot. 42:409-430. 1928.
2. BLAICKLEY, NELLIE M., The structure of the foot in certain mosses and in *Anthoceros laevis*. Trans. Roy. Soc. Edinburgh 57:699-709. 1932-1933.
3. BOLD, H. C., The nutrition of the sporophyte in the Hepaticae. Amer. Jour. Bot. 25:551-557. 1938.
4. ———, The nutrition of the sporophyte in the Musci. Amer. Jour. Bot. 27:318-322. 1940.
5. BOWER, F. O., Primitive land plants. London. 1935.
6. BUCH, H., Morphologie und Anatomie der Hepaticae. In VERDOORN'S Manual of bryology. The Hague. 1932.
7. CAMPBELL, D. H., The development of *Geothallus tuberosus* Campbell. Ann. Bot. 10:489-510. 1896.
8. ———, A remarkable development of the sporophyte in *Anthoceros fusiformis* Aust. Ann. Bot. 38:473-483. 1924.
9. CAVERS, F., A new species of *Riella* (*R. capensis* from South Africa). Rev. Bryol. 30:81-84. 1903.
10. DOUIN, CH., Le pédicelle de la capsule des Hépatiques. Bull. Soc. Bot. de France 55:194-202; 270-277. 1908.

11. GOEBEL, K., Archegoniatenstudien: Die einfachste Form der Moose. *Flora* 76:92-116. 1892.
12. ———, Archegoniatenstudien: Beiträge zur Kenntnis australischer und neuseeländischer Bryophyten. *Flora* 96:1-202. 1906.
13. ———, Organographie der Pflanzen. Part 2. Jena. 1930.
14. GYÖRFFY, I., Über Endorhizoiden von *Molendoa Hornschuchiana*. *Hedwigia* 49:101-103. 1910.
15. HOWE, M. A., and UNDERWOOD, L. M., The genus *Riella*, with descriptions of new species from North America and the Canary Islands. *Bull. Torr. Bot. Club* 30:214-224. 1903.
16. KIENITZ-GERLOFF, F., Vergleichende Untersuchungen über die Entwicklungsgeschichte des Lebermoos-Sporogoniums. *Bot. Zeitung* 32:193-203. 1874.
17. KÜSTER, E., Pathologische Pflanzenanatomie. Jena. 1925.
18. LANG, W. H., On the sporogonium of *Notothylas*. *Ann. Bot.* 21:201-210. 1907.
19. LEITGEB, H., Untersuchungen über die Lebermoose. IV. Graz 1879.
20. LORCH, W., Über die Saugzellen im Fusse und in der Vaginula bei den Lebermoosen. *Ber. deutsch. Bot. Ges.* 43:120-127. 1925.
21. ———, Über die Haustorialschläuche am Fusse der Laubmoose. *Ber. deutsch. Bot. Ges.* 43:262-270. 1925.
22. MEYER, K., Untersuchungen über den Sporophyt der Lebermoose. I. Entwicklungsgeschichte des Sporogons der *Corsinia marchantioides*. *Bull. Soc. Impér. Natural. Moscou* 1911:263-286. 1912.
23. ———, Untersuchungen über den Sporophyt der Lebermoose. II. Die Entwicklungsgeschichte des Sporogons bei *Plagiochasma*. *Bull. Soc. Impér. Natural. Moscou* 1913:597-615. 1914.
24. ———, Untersuchungen über den Sporophyt der Lebermoose. III. Das Sporogonium der *Corsinia marchantioides* Raddi. *Ber. deutsch. Bot. Ges.* 32:262-266. 1914.
25. O'KEEFE, LILLIAN, Structure and development of *Targionia hypophylla*. *New Phytol.* 14:105-116. 1915.
26. PORSILD, M. P., Sur une nouvelle espèce de *Riella* (subgen. nov.: *Trabutiella*) de l'Asie centrale. *Bot. Tidsskr.* 24:323-327. 1902.
27. STUDHALTER, R. A., Independence of sporophyte in *Riella* and *Sphaerocarpus*. *Ann. Bryol.* 11:153-154. 1938.
28. VRABER, M., Donos k poznavanju rodu *Riella*. *Prirod. Razp.* 2:125-164. 1933.
29. WATKINS, G. M., and WATKINS, M. O., A study of the pathogenic action of *Phymatotrichum omnivorum*. *Amer. Jour. Bot.* 27:251-262. 1940.

MACRO-ELEMENT NUTRITION OF THE TOMATO PLANT AS CORRELATED WITH FRUITFULNESS AND OCCURRENCE OF BLOSSOM-END ROT

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(WITH ELEVEN FIGURES)

Introduction

In the summer of 1941 an extensive experiment in plant nutrition was designed in order to examine the effects of varying concentrations of macro-nutrient elements in the nutrient medium on the ascorbic-acid content of tomato fruits. These results are reported elsewhere (8). Since blossom-end rot was particularly prevalent in certain treatments, it was of interest to collect and compile data on this disease. These data are reported in this paper.

Blossom-end rot of tomatoes was first reported by GALLOWAY (7), and the symptoms were described in some detail. Since then this disease has been recognized as occurring generally throughout the United States, Canada, Australia, New Zealand, and various parts of Europe. Some early investigators (12, 15, 17) attributed the pathic condition to the presence of one or more species of *Macrosporium* and *Fusarium* organisms, while SMITH (18) and others reported bacteria as the pathogen. Later FULTON (6) and BROOKS (2) verified the contention of SELBY (16) that no organisms were involved in the initial stages of the disease. More recently the occurrence of the rot has been correlated with such factors as soil moisture and water supply of the plant (3, 5, 9, 20, 21, 23), specific ion effects (9, 13, 25), and the osmotic concentration of the nutrient solution (9, 14). Modifying effects of nitrogen supply, carbohydrate content, length of day, light intensity, transpiration rates, bordeaux sprays, and varietal differences have also been reported. From the mass of more or less contradictory evidence which has accumulated, the viewpoint most widely accepted is that blossom-end rot is caused by a water deficit in the plant which results in a withdrawal of water from the fruit, with subsequent collapse of certain cells. As a result of this initial collapse, fungi or bacteria may be present in later stages of the disease.

Material and methods

The strain of Bonny Best variety of tomatoes used had been inbred for seven generations.⁴ Seed was planted in the greenhouse May 12, 1941, in 2-quart glazed

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⁴ Seed supplied through the courtesy of Dr. LEROY POWERS, U.S. Horticultural Station, Cheyenne, Wyoming.

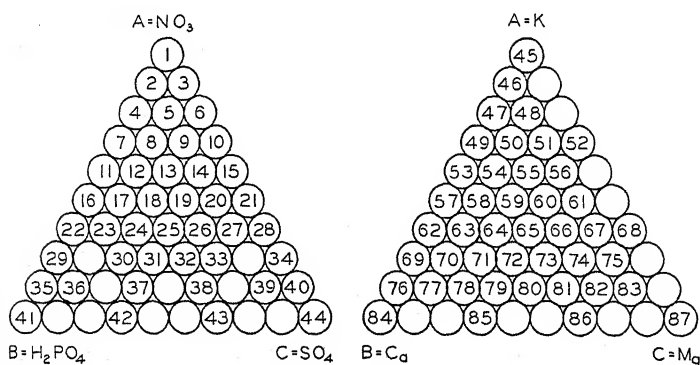
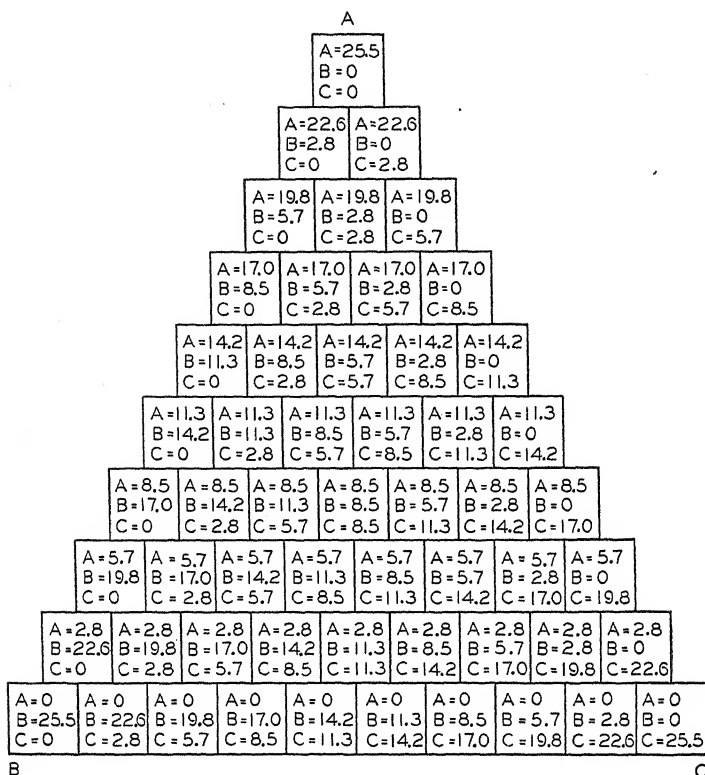


FIG. 1.—Upper triangle represents relative proportions of macro-nutrient elements in milliequivalents per liter for 55 possible treatments. Left, below: 44 combinations of varying anion concentrations. Right, below: 43 combinations of varying cation concentrations. Combinations indicated by treatment numbers.

crocks containing pure quartz sand. Two seeds were planted in each crock, and a complete nutrient solution (treatment 25, fig. 1) was used during germination and seedling stage. On June 9, when the plants were 28 days old and approximately 3 inches tall, uniform seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand, one seedling per crock. They were watered in with distilled water and immediately supplied with their respective nutrient treatments. On July 1 all plants were placed outdoors, the crocks being supported on wooden blocks. The plants were trained upright, both crocks and plants being supported by the stakes. All axillary growth was pruned off twice weekly. The plants were harvested September 10.

The design of the experiment was that of a randomized block (4), with eighty-seven treatments and four replications. Each replication consisted of a three-plant row, and the replications were randomized by the use of TIPPETT's randomization tables (22). Thus the mean of twelve plants is used as an estimation of the results produced by a given treatment. The data were reduced by means of the analysis of variance, and the *t* test (19) was used for determining whether particular differences were significant. Odds as great as or greater than 19:1 were accepted as statistically significant that the observed deviations were not due to the errors of random sampling. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained, both in the greenhouse and in the field.

The method of preparing nutrient solutions has been previously described (8). Each group of twelve plants was supplied subsequent to the seedling stage with one of eighty-seven different nutrient solutions which varied in the relative proportions of macro-nutrient elements. Thus a total population of 1044 plants was used. All solutions contained equal amounts of the micro elements in the following concentrations:

B as HBO_3	0.5 p.p.m.
Mn as MnCl_2	0.5
Zn as ZnSO_4	0.05
Cu as CuSO_4	0.02
Fe as ferric citrate.....	5.0

The relative proportions of potassium, calcium, and magnesium were varied in forty-four of these solutions, each of which contained 12.0 milli-equivalents per liter of nitrate, 4.5 m.e./l. of phosphate, and 9.0 m.e./l. of sulphate. In the remaining forty-three solutions the relative proportions of nitrate, phosphate, and sulphate were varied, while each contained 12.0 m.e./l. of calcium, 4.5 m.e./l. of potassium, and 9.0 m.e./l. of magnesium (fig. 1). For convenience, the nutrient solutions are represented in cation and anion triangles, and treatment numbers are

assigned to those solutions which were used. For purposes of discussion the treatments devoid in one or more ions will be designated as deficient.

The mean daily temperature for the duration of the experiment was 67.7° F., with a mean maximum of 80.9° and a mean minimum of 55.3° . The mean maximum temperatures in June, July, and August were 79.8° , 84.6° , and 79.3° F., respectively. The mean relative humidity from May 12 to September 10 was 45.2 per cent at 1:30 P.M. The average daily total of solar radiation for this period was 520.7 gram calories per square centimeter of horizontal surface.

Experimentation and results

On July 25 the plants were 74 days old and had received their respective nutrient treatments for 46 days. At this time the fruits on each plant were counted and the number recorded. The fruits showing blossom-end rot were immediately harvested and the percentage of total fruit per plant computed. Subsequent to this time the entire population was examined daily and any diseased fruits discarded. Fruit started to ripen in late July and early August, and each fruit was picked on the morning of the day that complete color change occurred. When picked, a fruit was immediately weighed. Comparable portions of the first two fruits to mature on each plant were dried at 60° C. for 4 days and selected chemical analyses made. Records were kept of the weight of each fruit as it was picked in order to compile data on total fruit production and mean fruit size. On September 10, when the plants were 121 days old and had received their respective nutrient treatments for 93 days, all fruits were picked, counted, and weighed.

Since the experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences, analyses of variance were computed for each individual character. The results are given in table 1. In characters A and B, one replication in one treatment was discarded as a result of mechanical injury, and YATES formula (24) was used for the estimation of the missing plot in order to maintain orthogonality.

It is evident (table 1) that statistically significant differences between treatments are inherent in the data. It is also evident that differences in environment when measured by replication produced significant differences in such characters as number of fruits set on July 25, mean number of fruits ripened per plant, and number and percentage of diseased fruit per plant. Since replication and randomization were essential to the design of the experiment, and since replication differences were measurable and not included in inter-treatment comparisons, valid differences exist which can be directly ascribed to treatments.

The data for the mean number of fruits set per plant on July 25, together with those for the occurrence of the rot at that time, are presented in figures 2 and 3. The greatest number of fruits set per plant in the anion triangle occurred in treat-

ments which contained 14.2-17.0 milliequivalents of nitrate per liter of nutrient medium. When lower concentrations of nitrate were used fruit set was correspondingly less, and when nitrate was omitted from the medium fruit set was negligible. When no phosphate was supplied to the plant subsequent to the seedling stage small numbers of fruits were obtained. In this experiment, however, 2.8 milliequivalents of phosphate was sufficient to result in a maximum expression of this character under conditions of comparatively high nitrate and low sulphate supply.

TABLE 1
RESULTS OF ANALYSES OF VARIANCE ON CHARACTERS
FOR WHICH DATA ARE REPORTED

CHARACTER	F VALUE	
	BETWEEN TREATMENTS*	BETWEEN REPLICATIONS†
A. Fruitfulness:		
1. Number set 7/25/41‡	29.21	24.00
2. Total number produced per plant	60.03	1.21
3. Total fresh weight	101.51	1.70
4. Mean number ripened per plant	31.85	5.50
5. Average fresh weight of all fruits (regardless of state of maturity)	44.62	1.51
6. Average fresh weight of mature fruits	33.45	0.85
7. Number of days from planting to date of first fruit ripe	11.91	0.79
B. Occurrence of blossom-end rot:		
1. Number of diseased fruits per plant 7/25/41	24.94	8.61
2. Percentage diseased per plant 7/25/41	14.01	8.77
C. Chemical analyses on first mature fruits:§		
1. Calcium	7.90 (1.63)	0.88 (2.72)
2. Potassium	37.44 (2.40)	2.36 (3.10)
3. Magnesium	9.34 (2.08)	0.79 (2.91)

* F value of 1.31 required for P value of 0.05: when $F = 1.49$, $P = 0.01$.

† F value of 2.64 required for P value of 0.05: when $F = 3.86$, $P = 0.01$.

‡ Subsequent to this time all fruits contracting the rot were harvested immediately.

§ For the chemical analysis, the F value required for P value of 0.05 is indicated in parentheses.

Of the plants grown in anion deficiencies, some sulphate-deficient treatments resulted in fruitful plants at this time. In treatment 22 fruitfulness is not significantly less than maximum, and concentrations of sulphate ranging from 2.8 to 11.3 m.e./l. were sufficient to result in maximum values in regard to this character.

In the anion triangle the rot, if it occurred at all, was not severe, and in most treatments there was no evidence of the disease. It is apparent that approximately 10 per cent of the fruits were diseased, in some of the treatments where maximum fruit set was observed. There was no trend which could be correlated with any anion effect.

The number of fruits set per plant at this time in treatments of the cation tri-

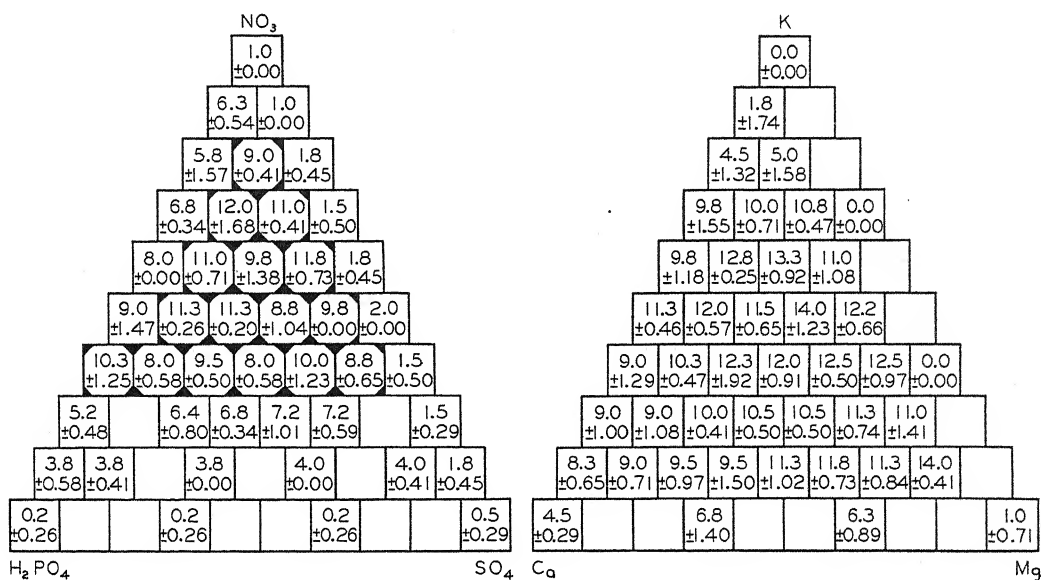


FIG. 2.*—Mean number of fruits set per plant on July 25 for anion (left) and cation (right) treatments.

* Treatment means, together with standard error. In any inter-treatment comparison, six degrees of freedom are available. When $t = 2.45$, $P = 0.05$. In this and all succeeding figures, results are given in triangular positions corresponding to equivalent positions in fig. 1.

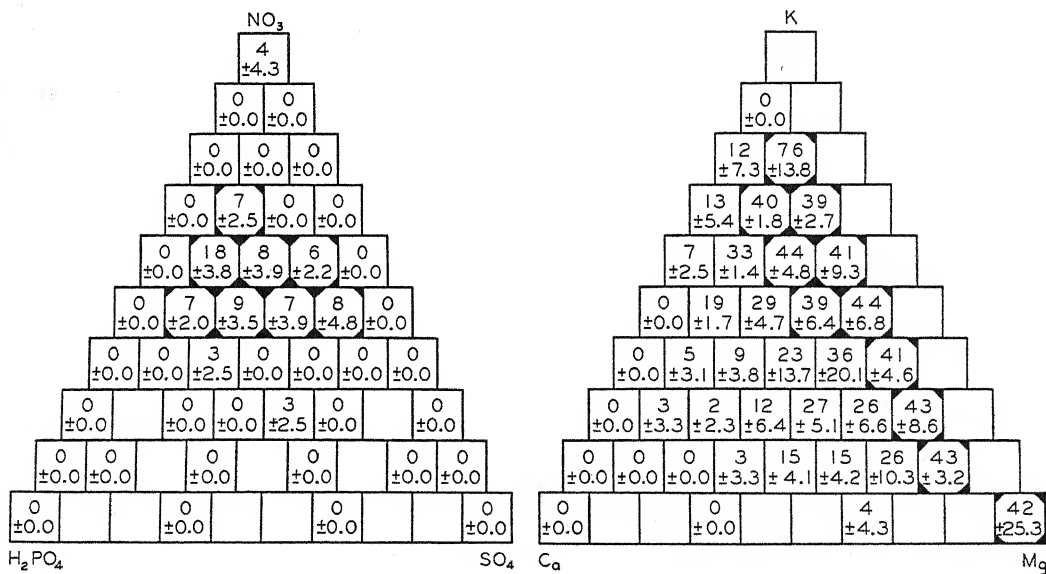


FIG. 3.—Percentage of diseased fruits per plant on July 25 for anion (left) and cation (right) treatments. See footnote to fig. 2.

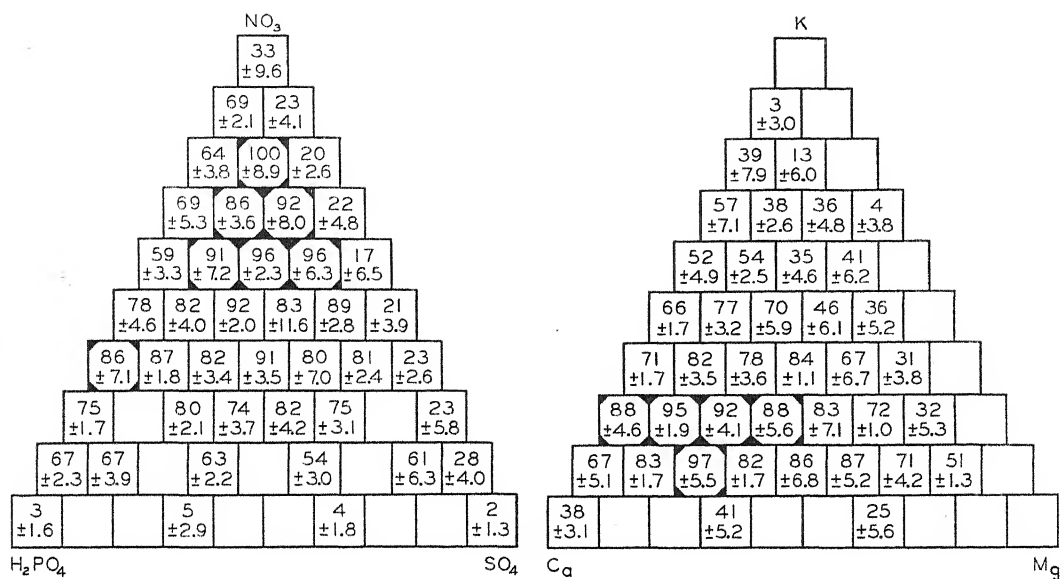


FIG. 4.—Average fresh weight of all fruit (grams) regardless of state of maturity at conclusion of experiment. Anion (left) and cation (right) treatments.

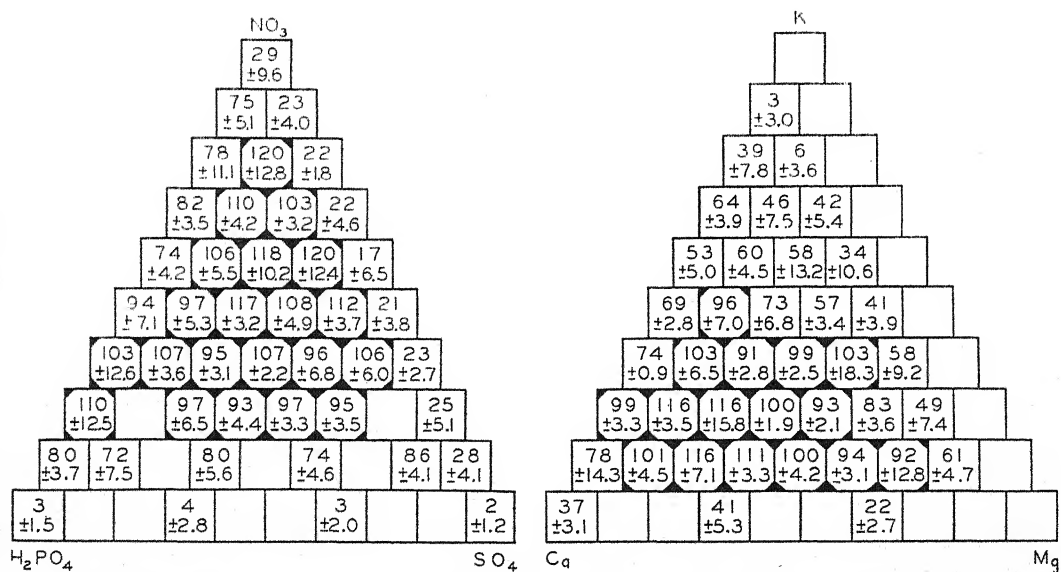


FIG. 5.—Average fresh weight of mature fruit only (grams) at conclusion of experiment. Anion (left) and cation (right) treatments.

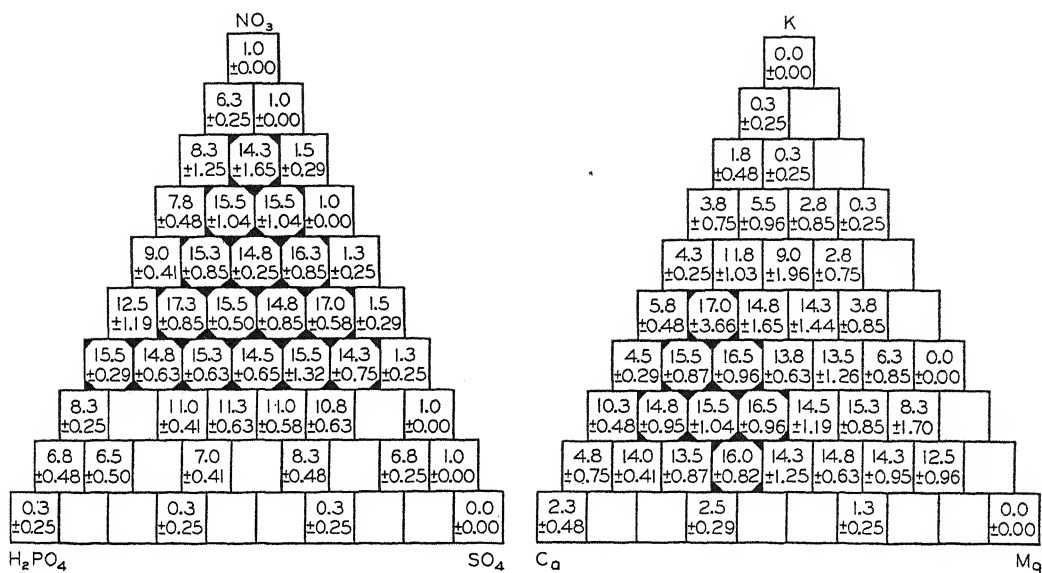


FIG. 6.—Total number of fruits regardless of state of maturity produced per plant at conclusion of experiment. Anion (left) and cation (right) treatments.

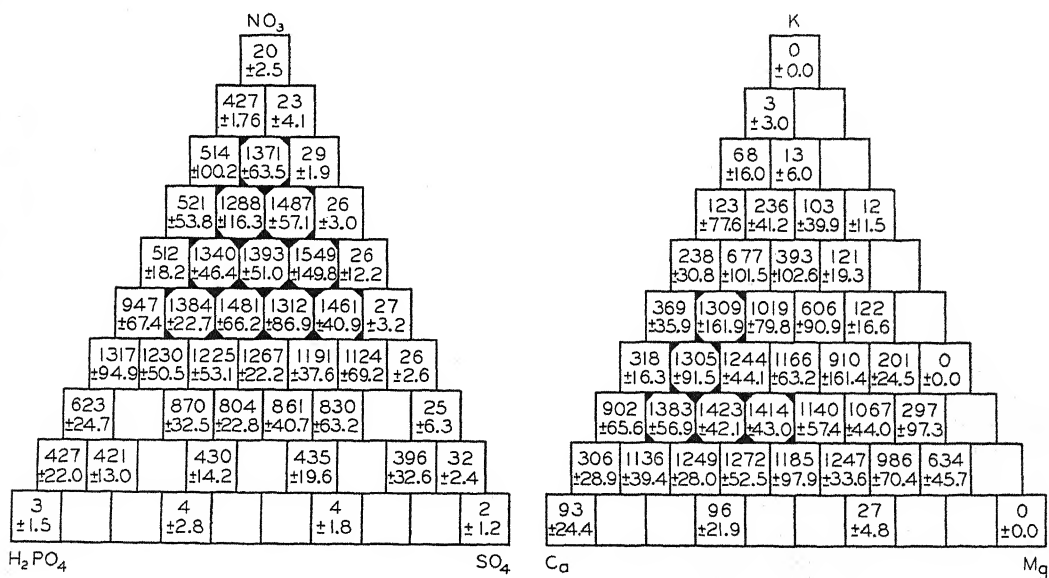


FIG. 7.—Total fresh weight of fruit per vine (grams) for anion (left) and cation (right) treatments

angle was not significantly affected by wide variations in relative composition of the nutrient medium. Significantly fewer fruits, however, were set in treatments deficient in one or more of the macro-nutrient cations. Since growing points were dead in calcium-deficient treatments, the number of fruits was less than in any other treatment. Fruit set was also significantly less than maximum in most of the potassium- and magnesium-deficient treatments, although some magnesium-deficient treatments are not affected in this respect. This observation will be discussed later.

The data for the occurrence of blossom-end rot in the cation triangle (fig. 3) showed a significant trend. As the concentration of calcium in the nutrient medium was less, the percentage of diseased fruits on each plant was correspondingly greater. In general, treatments containing 2.8 milliequivalents of calcium per liter of nutrient medium resulted in the greatest number of diseased fruit per plant and in the greatest percentage of diseased fruit per plant. There is an indication (treatments 48, 50, 55, 60) that relatively high concentrations of potassium and low concentrations of magnesium are associated with a greater percentage of diseased fruits at all calcium levels. This generalization is not applicable to magnesium-deficient treatments at comparable calcium concentrations, however, and the significance of the generalization is doubtful. If treatments deficient in one or more of the macro-nutrient cations are not considered, a significant correlation between the amount of calcium in the nutrient medium and the percentage of diseased fruit is demonstrable ($r = -0.90$, $t = 10.29$). The first diseased fruits occurred in treatments containing low concentrations of calcium. Subsequent to July 25, the greatest number of diseased fruits developed in the same treatments and accordingly were picked as the condition became apparent. In treatments containing greater amounts of calcium, the incidence of diseased fruits was correspondingly less subsequent to the initial harvest, and the diseased condition developed later than in those treatments represented by shaded areas in the triangles.

Additional data concerning fruitfulness are presented in figures 4-8. These data, which include the total weight and number of fruits produced, as well as fruit size, were compiled in an attempt to evaluate other characters of fruitfulness in so far as they could be correlated with the occurrence of the rot. The shaded portions of the triangles represent treatments where a maximum response was obtained for the character under consideration, and it is evident that, in general, the same treatments fall in the shaded portions for each character. These characters will be discussed together. The data for earliness (fig. 9) will be discussed separately.

In any interpretation of the data, it is well to note that all diseased fruits were harvested on July 25, and all fruits which set and were diseased subsequent to this time were immediately picked and discarded. Thus the following data pertain only to healthy fruits, and the data for the anion and cation triangles are discussed

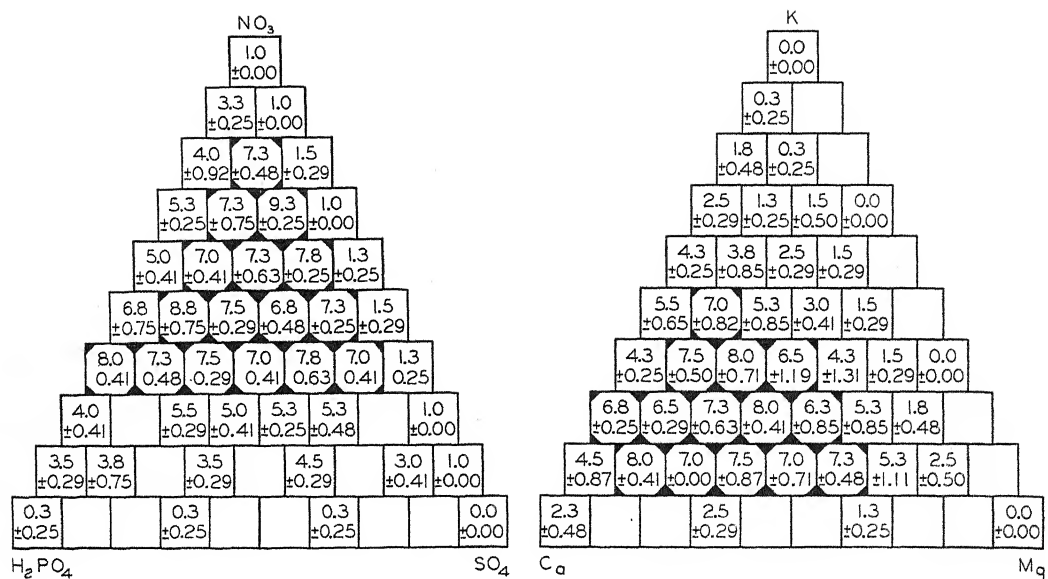


FIG. 8.—Number of fruit ripened per plant for anion (left) and cation (right) treatments

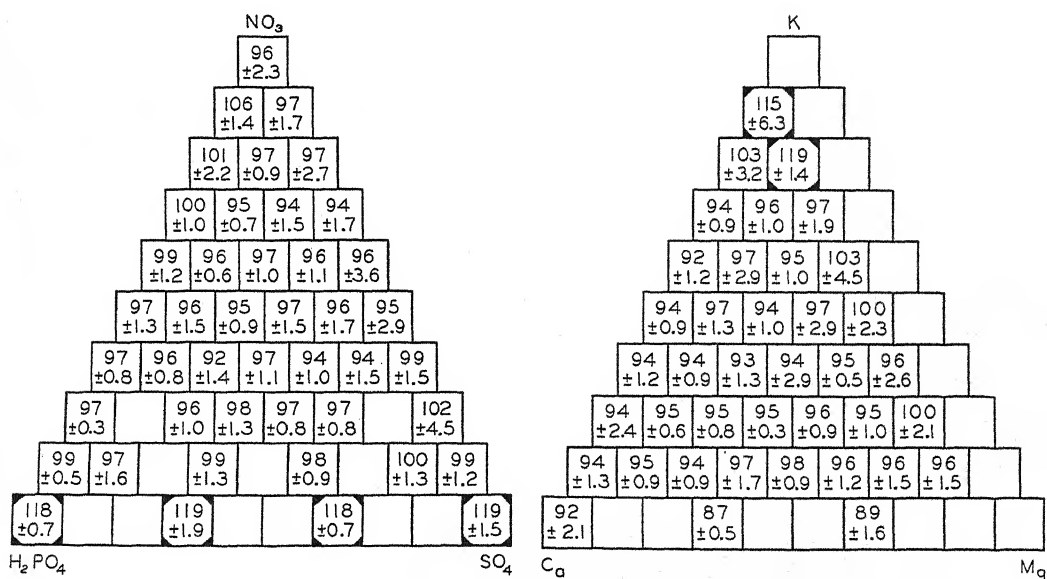


FIG. 9.—Number of days from planting to date of first fruits mature for anion (left) and cation (right) treatments

individually. In any comparison of figures 2 and 6, abscission at the pedicel joint of developing fruits is also a factor to be considered.

The trends in data for fruit characters in the anion triangle are uniform, and, in general, closely coincide with those previously discussed for the mean numbers of fruits set per plant on July 25. It should be emphasized, however, that these data were collected later in the life span of the plant, and the magnitude of the differences is increased. It is apparent that in those treatments where the rot was prevalent, maximum fruit characteristics were also observed.

In the cation triangle, however, the rot was not greatest in treatments which were the most fruitful. Relatively high concentrations of calcium (11.3–19.8 m.e./l.) are associated with maximum fruitfulness. When the concentration of calcium in the nutrient medium is less, fruitfulness is correspondingly less and the occurrence of diseased fruits correspondingly greater.

The validity of this correlation is further supported by the fact that the actual number of diseased fruits in low-calcium treatments (where fruitfulness was less) was greater than the number of diseased fruits in treatments supplied with greater amounts of calcium in the nutrient medium.

In so far as the date on which the first fruit ripened is concerned (fig. 9), wide variation in nutrient composition in the anion triangle produced no significant effect. When nitrate was absent in the media, however, the maturation of fruit was significantly slower. In the cation triangle the data for earliness showed little or no variation. There was indication, however, that a high potassium content in the nutrient medium inhibits fruit ripening and that a condition of potassium deficiency will hasten the ripening process. Fruits produced under these conditions are abnormally small.

Inasmuch as mineral composition of the nutrient medium may not be correspondingly reflected in the composition of fruits, chemical analyses are desirable to support or aid the interpretation of a correlation such as is presented here. Accordingly, analyses for the calcium content of fruits were made in certain treatments as indicated in figure 10, and the results of selected magnesium and potassium analyses are presented in figure 11.

Since diseased fruits were not available in all treatments, and since it is difficult to obtain such fruits at comparable stages of physiological development, the first healthy ripe fruit on each plant was selected for analysis. Such comparable fruits should reflect the conditions of the plant at a particular stage of physiological maturity. This condition would logically be associated with nutrient composition.

On the morning of the day that complete color change had occurred, the fruit was picked and immediately weighed. After a transverse section from the equatorial portion of the fruit was removed for vitamin analyses, the remaining apical and stem-end portions were weighed and dried at 60° C. for 96 hours. Such sam-

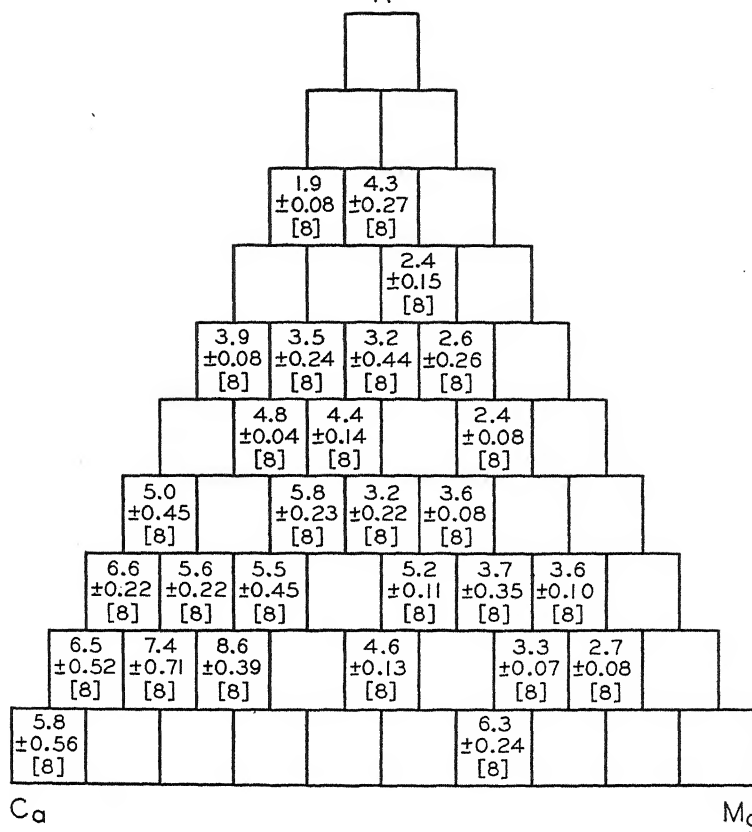


FIG. 10.—Calcium analyses on first healthy ripe fruit on each plant of treatments in cation triangle. Results in terms of milliequivalents per kilogram fresh weight of fruit. Number of analyses in each treatment indicated in brackets.

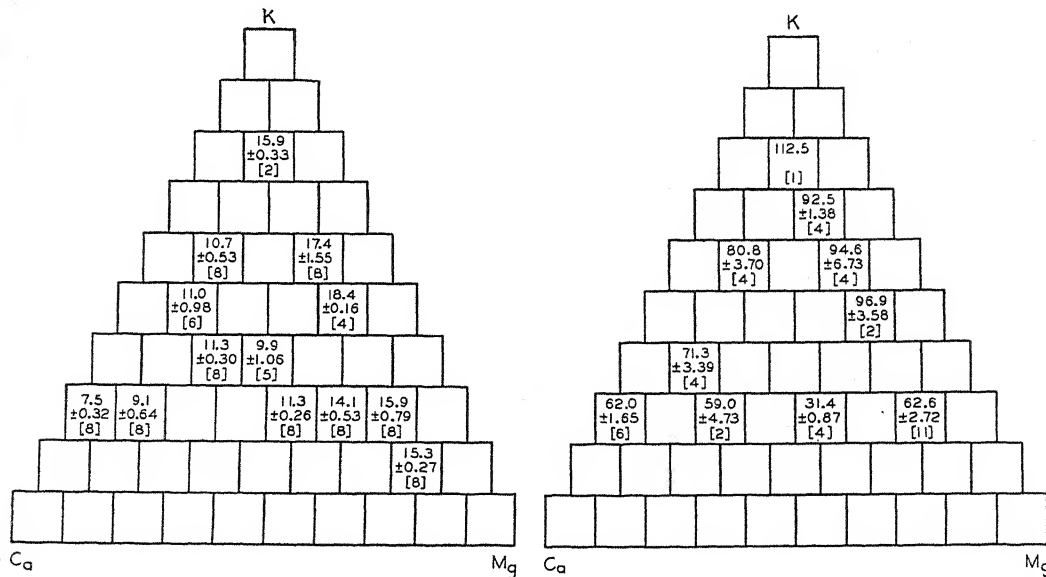


FIG. 11.—Same as fig. 10, except that analyses are for magnesium (left) and potassium (right)

ples included approximately 60–75 per cent of the fruit. The determinations of calcium, magnesium, and potassium were made according to the official methods of the A.O.A.C. (1), and the results are compiled in terms of milliequivalents of anion per kilogram fresh weight. The fruits analyzed were selected from treatments which represent wide variations in nutrient composition when all cations are considered.

From figure 10 it is apparent that, in general, a comparatively low concentration of calcium in the nutrient medium results in a low concentration of calcium in the fruit. Treatment 48 does not conform to this generalization, and it should be stressed that abnormally small fruit occurred at this point (figs. 4, 5). More than 10 per cent of the fruits were diseased in those treatments in which the first ripe fruits contained less than 5.5 milliequivalents of calcium per kilogram. Such relatively low concentrations of calcium were found in the fruit when the nutrient medium contained 2.8–11.3 milliequivalents of calcium per liter. The results of magnesium and potassium analyses (fig. 9) indicate comparatively high concentrations of both ions in fruits from treatments where the incidence of the disease was high. Although lower concentrations of both ions occurred in fruits from other treatments, a direct relationship with the occurrence of the rot was not apparent.

The ratios Mg:Ca and K:Ca were particularly high in the low calcium treatments (51, 56, 61, 75, and 83). This would be expected, when the nutrient composition is considered, and the significance of these ratios to diseased fruit is not therefore evident.

A considerable amount of magnesium is present in fruits of treatment 69. The plants of this treatment were supplied with no magnesium subsequent to the seedling stage. This condition may represent an accumulation in the first fruit of a portion of the magnesium which was supplied early in the life span of the plant. In fruits which subsequently matured, less magnesium would be expected. This hypothesis is being tested by analyses of the vegetative portions of the plant and of fruits which subsequently developed. A greater number of treatments, involving all the macro-nutrient elements, are under consideration in an attempt to clarify the influence of the relative composition of the nutrient medium on ion absorption. The results will be presented in a later paper.

Discussion

When the characters listed in table 1 are used as criteria of fruitfulness, it is apparent that maximum responses are obtained in a definite group of treatments and that optimum nutrient composition in this experiment can be designated within narrow limits. In general, variations in calcium and nitrate produced more apparent differences over wider ranges in concentrations than were obvious with other macro-nutrient elements. Greatest fruitfulness occurred when the concen-

tration of calcium in the nutrient medium was comparatively high. Similarly, as the concentration of nitrates was increased from 0.0 to 8.5 m.e./l., fruitfulness was correspondingly greater. It is recognized, however, that nutrient composition which is optimum for fruitfulness may not be optimum for such characters as height of plant and fresh and dry weight of root system.

The interaction of certain ions is obvious. Thus, at concentrations of calcium of 5.7 milliequivalents, greater fruitfulness resulted when potassium was relatively low and magnesium relatively high. Other examples could be cited, and the interactions are particularly apparent in treatments completely lacking one or more ions.

The occurrence of blossom-end rot is clearly correlated with calcium nutrition. In the cation triangle, with decreasing concentrations of calcium in the nutrient medium the percentage of diseased fruits was correspondingly greater. The calcium content of fruits was associated with the amount of calcium supplied to the plant. Fruits which contained the smallest amounts of calcium were produced in treatments where the disease was most prevalent. No correlation of the occurrence of this rot with any other macro-nutrient element was observed. The percentage of diseased fruits in certain treatments of the anion triangle closely approximated values for treatments of similar nutrient composition in the cation triangle. Thus treatment 19 in the anion triangle, which closely corresponds to treatment 72 in the cation triangle in so far as nutrient composition is concerned, shows the same percentage of diseased fruits within the limits of error (7 ± 3.9 and 12 ± 6.4 , respectively).

The results reported here are in agreement with the observations of RALEIGH (13), using aerated nutrient culture solution with and without calcium. YOUNG (25) observed relatively less blossom-end rot with the application of potassium fertilizers to soils. The data reported here show no primary effect of potassium in sand culture which would support this viewpoint. HOFFMAN (10) reports that the application of potash fertilizers to soils increases the occurrence of the rot only to a limited degree. This is in agreement with the present data.

It is recognized that there is no simple explanation for the occurrence of this disease. However, two related factors, such as calcium nutrition and moisture relations of the plant, might be used as a basis for the correlation of other observations.

Whether one of these factors functions independently of the other is a question not answered in the literature. In this experiment it might be reasoned that root development in pot culture is possibly not comparable with soil conditions. Such restricted root development might result in less water absorption by the plant. If this were the only factor involved, it seems probable that all variations in nutrient supply would result in the same percentage of diseased fruits. It might

further be reasoned that when a relatively small amount of calcium was supplied in the nutrient medium, less extensive root growth—and hence less water absorption—occurred. That this hypothesis is untenable is shown by the fact that both vine and root growth, as measured by fresh- and dry-weight production, are greatest in low calcium treatments (2.8 milliequivalents of calcium per liter of nutrient medium). Furthermore, the percentage dry matter of the vine is significantly less in low calcium treatments as compared with high. These data would thus indicate that those treatments where the rot was most prevalent resulted in the most succulent vines and the most extensive root systems. Thus, in this experiment water intake was actually relatively greater in low calcium treatments.

Since no direct effect of potassium was observed in sand culture where exchange phenomena are absent, it would seem possible that a secondary effect is being observed in cases where potash applications to soils reduce the occurrence of the disease. If one were dealing with a very acid soil with a relatively small quantity of calcium in the colloidal complex, it is conceivable that an increase in the concentration of potassium ions in the soil solution would liberate some calcium in the exchange complex, making it more readily available to the plants.

KERTESZ *et al.* (11) have reported that the addition of 170 p.p.m. of calcium chloride to peeled tomatoes for a short time during the canning process will result in firmer fruits with no subsequent disintegration of fleshy portions. Their data show that an appreciable amount of calcium has been absorbed by the cells and is not subsequently lost by drainage. The drained weight is materially increased over that of untreated tomatoes. Their data seem to indicate a greater retaining capacity of cells for water or soluble materials.

The role that calcium has in water relationships within the plant is not at all clear, and the following questions might be proposed: (a) Does calcium in the cell walls or in semipermeable membranes influence the water-retaining capacity of cells? If so, an adequate accumulation in the fruit is necessary to prevent water withdrawal. Also, a lack of calcium in the fruit might result in a "flooding" of intercellular spaces, thereby limiting gaseous exchange for metabolism. (b) Is water transfer controlled by or related to the content of calcium in the cell walls? If so, do cells "compete" for water supply? The following questions might also be considered concerning calcium in the external environment: (a) Does an "imbalance" of the nutrient media resulting from variations in calcium supply result in differential ionic absorption? This may be indicated in the potassium and magnesium analyses of fruits in this experiment. (b) Is the volume of nutrient supply a factor in the absorption of water and nutrient elements?

While these questions are in no way clarified by the data presented here, their importance to an understanding of the disease is suggested.

Summary

1. A population of 1044 plants of an inbred strain of Bonny Best tomatoes was grown in sand culture. The effects of eighty-seven different nutrient solutions varying in the relative proportions of macro-nutrient elements were studied in relation to fruitfulness and the occurrence of blossom-end rot. The data were reduced and analyzed by statistical methods.

2. In general, variations in amounts of calcium and nitrate in the nutrient medium resulted in greater differences in fruitfulness over wider ranges in concentrations than did the other elements.

3. Greatest fruitfulness occurred in treatments relatively high in nitrate and low in sulphate and phosphate in the anion triangle and in treatments relatively high in calcium and low in magnesium and potassium in cation triangle.

4. The percentage of diseased fruits on each plant increased with decreasing calcium concentrations in the nutrient medium. This correlation is largely independent of magnesium and potassium concentrations, and no correlation with any anion was observed.

5. Fruits produced in treatments where the rot was most severe were low in calcium content and high in potassium and magnesium content.

6. The occurrence of the rot was definitely associated with calcium nutrition.

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LITERATURE CITED

1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.—Methods of analysis. 5th ed. 1940.
2. BROOKS, C., Blossom-end rot of tomatoes. *Phytopath.* 4:345-373. 1914.
3. CHAMBERLAIN, E. E., Blossom-end rot of tomatoes. *Jour. Agr. New Zealand* 46:293-296. 1933.
4. FISHER, R. A., *The design of experiments*. London. 1937.
5. FOSTER, A. C., Effect of environment on the metabolism of the tomato plant as related to development of blossom-end rot of the fruit. *Phytopath.* 29:7. 1939.
6. FULTON, H. R., WRIGHT, W. J., and GREEG, J. W., The control of insects and diseases affecting horticultural crops. *Pennsylvania Agr. Exp. Sta. Bull.* 110. 39. 1911.
7. GALLOWAY, B. T., Notes on the black-rot of the tomato. *U.S. Dept. Agr. Rept.* 1888. 339-346. 1888.
8. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Factors influencing the ascorbic-acid content of the tomato with special reference to mineral nutrition. *BOT. GAZ.* 103: 586-616. 1942.
9. HAYWARD, H. E., Unpublished data; private communication.
10. HOFFMAN, J. C., The relation between water deficiency and blossom-end rot of tomatoes. Abstracts of doctor's dissertations, no. 24. Ohio State University Press. 1937.
11. KERTESZ, Z. I., TOLMAN, T. G., LACONTI, J. D., and RUYLE, E. H., The use of calcium in the commercial canning of whole tomatoes. *N.Y. (Geneva) Agr. Exp. Sta. Tech. Bull.* 252. 1940.

12. QUINN, G., The production of early tomatoes. South Australia Dept. Agr. Bull. 6:15. 1905.
13. RALEIGH, G. J., Fruit abnormalities of tomatoes grown in various culture solutions. Proc. Amer. Soc. Hort. Sci. 37:895-900. 1939.
14. ROBBINS, W. R., Relation of nutrient salt concentration to growth of the tomato and to the incidence of blossom-end rot of the fruit. Plant Physiol. 12:21-50. 1937.
15. ROLFS, P. H., The tomato and some of its diseases. Florida Agr. Exp. Sta. Bull. 21. 36. 1893.
16. SELBY, A. D., Investigations of plant diseases in forcing house and garden. Ohio Agr. Exp. Sta. Bull. 73. 241-242. 1897.
17. SMITH, E. H., Blossom-end rot of tomatoes. Massachusetts Agr. Exp. Sta. Tech. Bull. 3. 1907.
18. SMITH, R. E., Report of the Plant Pathologist to July 1, 1906. California Agr. Exp. Sta. Bull. 184. 244. 1907.
19. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
20. STOUT, G. J., Influence of watering treatment on the occurrence of blossom-end rot in greenhouse tomatoes. Proc. Amer. Soc. Hort. Sci. 32:515-518. 1934.
21. STUCKEY, H. P., and TEMPLE, J. C., Tomatoes. II. Blossom-end rot. Georgia Agr. Exp. Sta. Bull. 96. 69-91. 1911.
22. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge Univ. Press. London. 1927.
23. WEDGWORTH, H. H., NEAL, D. C., and WALLACE, J. M., Wall and blossom-end rot of the tomato. Mississippi Agr. Exp. Sta. Bull. 247. 1927.
24. YATES, F., The empire journal of experimental agriculture. 1:129. 1933.
25. YOUNG, P. A., HARRISON, A. L., and ALTSTATT, G. E., Common diseases of tomatoes. Texas Agr. Exp. Sta. Circ. 86:1-32. 1940.

RELATIONSHIP OF DISSOCIATION OF CELLULAR PROTEINS BY AUXINS TO GROWTH¹

HENRY T. NORTHEN

(WITH ONE FIGURE)

Protoplasm is a system which, like *in vitro* protein ones, may be reversibly dissociated by various agents (52). The data presented in this paper indicate that auxins condition a dissociation of cellular proteins as evidenced by decreases in the structural viscosity of protoplasm. Such dissociation, with a concomitant decrease in the molecular weight of cellular proteins and an increase in the number of active sulfhydryl groups, may condition accelerated rates of imbibition, diastatic activity, and respiration, as well as modify development.

Material and methods

Stems and petioles from navy bean plants 8-15 days old, which had been grown in window boxes of the laboratory, were smeared unilaterally with hydrated lanolin preparations of indole-3-acetic acid, indole-3 α -propionic acid, and α -naphthalene acetic acid. After the desired periods (tables 1-7), the responses were noted. Then a 1.5-cm. piece from each stem or petiole was centrifuged between wet cotton with an acceleration of 680 \times gravity for 4 minutes, an acceleration which would not displace chloroplasts or starch grains in most cells of control petioles or stems. In the centrifuge tubes the axes of the plant parts were at right angles to the direction of the acceleration. Following centrifugation, freehand cross-sections were prepared and mounted in IKI. Next the percentages of cells in which the chloroplasts or starch grains were displaced on the auxin-treated sides and on the control sides were determined. In other experiments the tops of decapitated plants were daubed with lanolin or with lanolin containing growth substance. Such stems were then centrifuged, sectioned, mounted, and observed. About 4000 plants were treated, of which percentages of displacement were determined for about 500 plant parts. Approximately 150,000 cells were counted.

Data

PETIOLES TREATED ON ONE SIDE

MIXTURE OF 1 MG. INDOLE-3-ACETIC ACID IN 1 GM. LANOLIN.—In table 1, data for a typical experiment, one of three, are recorded. It will be noted that with all

¹ Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 189.

TABLE 1

HOURS EXPOSED	RE- SPONSE	PERCENTAGE OF CELLS SHOWING DISPLACEMENT IN			
		CORTEX		RAY	
		I.A. SIDE	CONTROL SIDE	I.A. SIDE	CONTROL SIDE
0.0.....			3		0
0.0.....			2		0
0.5.....	0*	65	15	62	28
0.5.....	0	52	14	36	9
1.0.....	—	68	16	65	5
1.0.....	—	70	9	68	9
1.5.....	—	89	30	92	37
1.5.....	—	89	20	94	39
2.0.....	—	92	21	84	16
2.0.....	—	87	30	79	23
3.0.....	—	90	28	99	45
20.0.....	—	66	18		
20.0.....	—	71	16		

* In this and following tables: 0, none; —, growth greater on i.a. side; +, growth greater on control side.

TABLE 2

CONCENTRATION (MG. PER GM.)	HOURS EXPOSED	RESPONSE	PERCENTAGE OF COR- TEX CELLS SHOWING DISPLACEMENT OF CHLOROPLASTS ON	
			I.P. SIDE	CONTROL SIDE
20	20.0.....	0	12	7
	20.0.....	0	26	18
	22.0.....	0	2	2
40	3.0.....	—	67	14
	3.5.....	—	64	11
	4.5.....	—	54	16
	5.5.....	—	80	10
	6.5.....	—	86	34
60	2.0.....	—	75	8
	5.0.....	—	81	9
	6.0.....	—	90	27
	6.5.....	—	84	15
	28.0.....	—	96	17
100	0.2.....	0	5	3
	0.5.....	—	71	7
	1.0.....	—	80	27
	1.5.....	—	75	11
	3.0.....	—	51	15
	5.0.....	+	6	85

exposures the chloroplasts in the cortical cells and the starch grains in the ray cells were displaced by the centrifugation in greater percentages of cells on the indole-3-acetic acid (i.a.) side than on the control side. This indicates that the structural viscosity of protoplasm has been decreased on the i.a. side. Presumably decreases in structural viscosity are conditioned by a dissociation of cellular proteins.

INDOLE-3N-PROPIONIC ACID.—In all instances (table 2) the structural viscosity was lower on the convex than on the concave side in the experiments with this acid (i.p.).

MIXTURE OF 1 MG. α -NAPHTHALENE ACETIC ACID IN 1 GM. LANOLIN.—The data from one of three experiments which agreed closely are recorded in table 3. Dur-

TABLE 3

HOURS EXPOSED	RESPONSE	PERCENTAGE OF CORTEX CELLS SHOWING DISPLACEMENT OF CHLOROPLASTS ON		HOURS EXPOSED	RESPONSE	PERCENTAGE OF CORTEX CELLS SHOWING DISPLACEMENT OF CHLOROPLASTS ON	
		N.A. SIDE	CONTROL SIDE			N.A. SIDE	CONTROL SIDE
0.0.....			5	3.0.....	—	55	10
0.0.....			11	3.0.....	+	82	81
0.5.....	—	25	4	3.0.....	+	82	80
0.5.....	—	54	21	4.5.....	—	93	47
1.0.....	—	48	7	4.5.....	—	89	26
1.0.....	—	65	24	4.5.....	+	67	72
1.5.....	—	61	12	4.5.....	+	69	53
1.5.....	—	78	7	24.0.....	—	57	30
3.0.....	—	91	30	24.0.....	—	62	14

ing the initial negative curvature the viscosity was lower on the faster than on the slower growing side. Three hours and $4\frac{1}{2}$ hours after application of the n.a., some petioles exhibited a negative bending and others a positive. In the former instance the viscosity was lower on the auxin side. When the curvatures were positive, however, the viscosity was low on both sides. At the end of 24 hours all the petioles exhibited a negative bending and the viscosity was lower on the auxin side.

PETIOLES TREATED ON UPPER OR LOWER SURFACE WITH 1 MG. OF
 α -NAPHTHALENE ACETIC ACID PER GM. OF LANOLIN

In this experiment percentages of displacement were determined in various areas of the cortex. In table 4 the regions are indicated by numbers. The diagram

(fig. 1) reveals the area of the petiole corresponding to each number. Regions three and six are those of collenchyma, whereas the others are of chlorenchyma. Each percentage is the average of fifteen sections cut from five petioles.

TABLE 4

HOURS EXPOSED	RE- SPONSE	SURFACE TREATED	PERCENTAGE DISPLACEMENT IN REGION					
			1	2	3	4	5	6
0.5.....	—	Upper	54	41	0	2	14	0
0.5.....	—	Lower	3	7	8	15	51	88
1.0.....	—	Upper	70	78	55	32	16	34
1.0.....	—	Lower	8	18	75	70	65	75
1.25.....	—	Upper	64	70	78	8	11	0
1.25.....	—	Lower	0	4	43	53	91	96
2.0.....	—	Upper	31	45	45	37	41	43
2.0.....	—	Lower	40	38	68	78	89	94
3.5.....	+	Upper	58	78	90	73	68	92
3.5.....	+	Lower	84	89	60	82	70	48

Initially the viscosity was decreased only in regions near the point of application. With longer exposures the viscosity was decreased in more distant regions, until at the end of $3\frac{1}{2}$ hours it was about the same in remote locations as in close ones. With the exception of the experiment where the upper surface was treated and the exposure was 2 hours, increased growth was paralleled by lowered viscosity when the curvatures were negative. When the bendings were positive, however, the differences between the two sides were not significant.

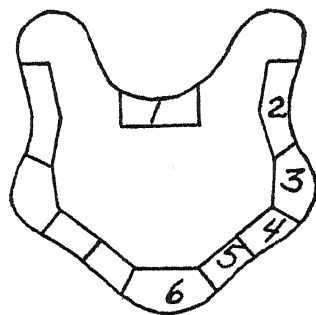


FIG. 1

AUXINS APPLIED UNILATERALLY TO FIRST INTERNODE OF STEMS

MIXTURE OF 1 MG. INDOLE-3-ACETIC ACID IN 1 GM. OF LANOLIN.—The protoplasmic viscosity was decreased in ray cells on the i.a. side before any noticeable bending occurred (table 5). When the curvature was negative the viscosity was lower on the auxin than on the control side. After 2 hours there was little difference in the protoplasmic viscosity of the two sides.

INDOLE-3-N-PROPIONIC ACID.—In general, the data (table 6) indicate that when curvatures are negative the structural viscosity of the protoplasm is lower on the convex side.

AUXINS APPLIED ON TOPS OF FIRST INTERNODES OF STEMS

MIXTURE OF 10 MG. INDOLE-3N-PROPIONIC ACID IN 1 GM. OF LANOLIN.—In this experiment plants were decapitated just below the first pair of leaves. The cut surfaces of some of the plants were then daubed with lanolin and those of others with a mixture of lanolin and i.p. acid. Following centrifugation, sections were

TABLE 5

HOURS EXPOSED	RESPONSE	PERCENTAGE OF RAY CELLS SHOWING STARCH GRAINS DISPLACED	
		I.A. SIDE	CONTROL SIDE
0.0.....	3
0.0.....	5
0.5.....	o	60	17
0.5.....	o	54	3
1.0.....	—	62	9
1.0.....	—	81	24
2.0.....	+	92	94
2.0.....	+	83	79

TABLE 6

CONCENTRATION (MG. PER GM.)	HOURS EXPOSED	RESPONSE	PERCENTAGE DISPLACEMENT IN			
			CORTEX CELLS		RAY CELLS	
			I.P. SIDE	CONTROL SIDE	I.P. SIDE	CONTROL SIDE
20	0.0.....	3	5
	5.0.....	—	86	32	84	17
	0.0.....	o	o
60	1.0.....	—	10	10	33	11
	1.5.....	—	80	10	42	21
	24.0.....	—	72	10	96	38

cut at different distances from the surface. At the end of 22 hours slight swellings were noted at the tops of those plants which received this acid. Such protuberances subsequently increased in size. Their behavior was very much like that described by KRAUS, BROWN, and HAMNER (36). The experiment involving exposures of 11 and 12 hours was performed when the internodes were 3 cm. long, whereas the other experiment, involving 22- and 28-hour exposures, was per-

formed when the internodes were 4 cm. long. The data (table 7) indicate that i.p. acid conditions a decrease in structural viscosity.

MIXTURE OF 1 MG. α -NAPHTHALENE ACETIC ACID IN 1 GM. OF LANOLIN.—After an exposure of 22 hours the starch grains were displaced by centrifugation in 76 per cent of the experimental ray cells (5 mm. from the apex) and in 11 per cent of the control ray cells.

TABLE 7

HOURS EXPOSED	MILLIMETERS FROM APEX	PERCENTAGE OF DISPLACEMENT IN					
		CORTEX CELLS		RAY CELLS		PITH CELLS	
		LANOLIN +I.P.	LANOLIN ONLY	LANOLIN +I.P.	LANOLIN ONLY	LANOLIN +I.P.	LANOLIN ONLY
11	{ 3.....	70	89	100
	{ 7.....	92	14	93	28	100	30
	{ 10.....	90	100	100
12	{ 3.....	85	92
	{ 7.....	83	9	100	59	100	44
	{ 10.....	86	91	97
22	{ 3.....	70	0	55	0	86	0
	{ 7.....	100	0	66	0	100	0
28	{ 3.....	56	79	100
	{ 7.....	55	19	80	22	100	9
	{ 10.....	70	90	100

INDOLE-3N-PROPIONIC ACID APPLIED ON TOPS OF SECOND INTERNODES OF STEMS

The tops of some second internodes were treated with lanolin and others with lanolin containing 10 mg. of i.p. acid per gram. Following centrifugation, sections were cut 4 mm. from the apex. Displacements of starch grains were noted in 85 per cent of the ray cells from plants which received this acid and in 1 per cent from control plants after 5 hours' exposure, in 76 and 0 per cent, respectively, after 10 hours and in 80 and 0 per cent, respectively, after 24 hours, thus manifesting that the structural viscosity had been decreased by the acid.

Discussion

The application of growth substances to plants may condition increased plasticity of cell walls (26), intussusception of new wall material (68), augmented swelling pressure (72), quickened rates of respiration (1, 8, 58, 75, 81) and carbohydrate hydrolysis (5, 48), initiation of cell division (7, 28, 47, 67, 79), and at times mitotic abnormalities (20, 36).

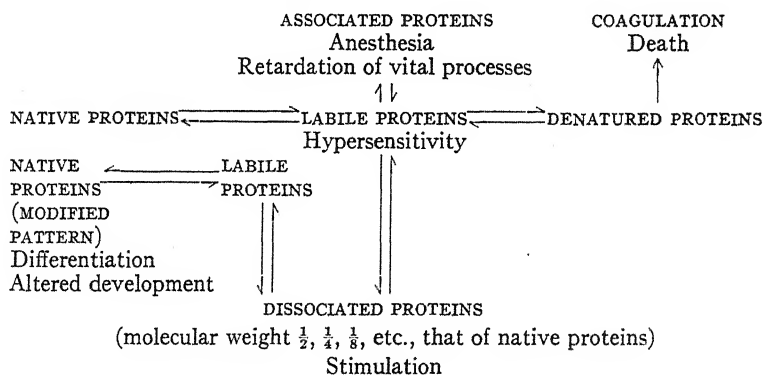
With the possible exception of wall plasticity, all the listed processes are under protoplasmic control. The rates of such processes are often increased by agents,

including growth substances, which condition a dissociation of cellular proteins. For example, carbon dioxide, anesthetics, disease, alternating currents, direct currents, warm baths, wounding, X-irradiation, soap, and sodium taurocholate initially cause a dissociation of cellular proteins, as evidenced by decreases in viscosity (3, 13, 25, 49, 50, 51, 53, 54).

Of these the following may initially increase the rate of respiration: carbon dioxide (76, 77), anesthetics (21, 34), disease (59), mechanical agents (4, 19, 31), sodium taurocholate (10), and X-irradiation (66). The hydrolysis of starch may be accelerated by carbon dioxide (77), anesthetics (33), alternating currents (40), warm baths (33), wounding (31), and X-irradiation (66). Some agents which initiate cell division in unfertilized animal eggs are carbon dioxide (27), ether (45), electrical currents (14), warm baths (44), and soaps (42). Agents which dissociate cellular proteins and produce mitotic abnormalities are anesthetics, high temperatures, wounding, disease, and X-irradiation (65).

The data presented indicate that growth substances condition a dissociation of cellular proteins. In this particular they act like many agents. It has been suggested that such dissociation conditions accelerated rates of many processes which are controlled by protoplasm.

The following diagram illustrates some of the transmutations which may occur when cellular proteins are subjected to environmental changes. The physiological resultants of each state are also listed.



Rates of following are usually increased:

1. Swelling
2. Respiration
3. Carbohydrate hydrolysis
4. Permeability

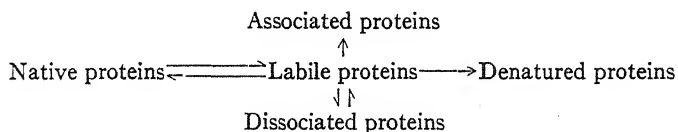
It must be emphasized that the series of reactions is not entirely hypothetical but is based on experimental data. NORTHERN (51) obtained evidence which suggested that labile proteins were formed prior to dissociation. He noted that solu-

tions of ether, so dilute that neither dissociations nor associations resulted, rendered the protoplasmic proteins more sensitive to dissociation by a mechanical impact. Apparently the dilute ether changed the native proteins into the more readily dissociable labile proteins.

Previously data have been cited which indicate that many agents decrease the structural viscosity of protoplasm. It is probable that decreases in structural viscosity are conditioned, in part at least, by dissociations of cellular proteins. First, because agents which decrease the structural viscosity of protoplasm dissociate *in vitro* proteins. The following agents which decrease protoplasmic viscosity may dissociate proteins: temperature of 35° C. (69), sound (11), amides (22, 71), alcohols (70), and X-irradiation (6). Second, because viscosity may be proportional to molecular weight (STAUDINGER, in SCHMIDT, 64).

When cells are treated with some agents an increase in viscosity follows an initial decrease (3, 25, 49, 52, 53). It is likely that the increase is conditioned in part by an association of cellular proteins.

The concept of protoplasm as a reversibly dissociable-associable system is in harmony with biochemical evidence. The preceding diagram was modeled from that suggested by LUNDGREN and WILLIAMS (43) for *in vitro* proteins. They outlined the series as follows:



Prior to them, SØRENSEN (70) and SVEDBERG (73) concluded that proteins were reversibly dissociable-associable component systems. SVEDBERG states: "The lability [of proteins] reveals itself in a number of well defined and reversible dissociation-association reactions, the causes of which are changes in the environment." In other words, even *in vitro* protein systems are irritable. They can respond to relatively small environmental changes.

According to the dissociation-association idea of protoplasm, the dissociation of cellular proteins, by auxins or other agents, conditions an increased swelling pressure, which is probably a factor in the enlargement of cells. Biochemical evidence supports this deduction. NORTROP (55) noted that a gel which ceased to take up water again did so if the network lost its elasticity. LLOYD (41), from studies on the relationship between protein structure and water absorption, concluded that the more compact the structure the less the swelling and the looser the structure the greater the swelling. KUTHY (38) observed that substances which tended to change gels into sols promoted swelling, whereas those which conditioned the reverse reaction inhibited swelling. Moreover, from a thermodynamical point

of view, and in accordance with the data presented, it is highly probable that increased swelling is conditioned by a dissociation of cellular proteins.

The swelling pressure of a gel (and presumably of a structural system like protoplasm) is related to vapor pressure and molecular weight according to the following equation (64, p. 471):

$$P = -\frac{RT}{MV_0} \ln h$$

in which P is the swelling pressure, R the gas constant, T the absolute temperature, M the molecular weight, V_0 the specific volume of the liquid, and h the relative vapor pressure (the ratio of the vapor pressure of the gel to that of the pure solvent). Hence, if an agent decreases the molecular weight of cellular proteins or the vapor pressure of the protoplasm, the swelling pressure will increase. Accordingly, if water be present and the walls are plastic, cells may enlarge. The vapor pressure of protoplasm may be lowered when cellular proteins dissociate through the splitting of proteins, the liberation of ions, sugars, and other substances from their previous combinations, and by the disengagement and activation of hydrolyzing enzymes.

The diminution of the molecular weight of proteins, conditioned by auxins and other agents, may greatly augment the protoplasmic swelling pressure. According to the equation, if the molecular weight be halved the swelling pressure will be doubled; if quartered, quadrupled; and so on. That proteins may be strikingly dissociated is demonstrated by the work of HAND (22), who noted that the particle weight of urease was reduced from 700,000 to 17,000 by urea. If such a decided dissociation occurred when cellular proteins were disaggregated by auxins or other agents, the swelling pressure would be increased more than forty-one times.

The previously presented data demonstrate that in many instances cell enlargement is correlated with dissociation. In some cases the relationship may or may not hold, particularly where positive curvatures were noted and when the viscosity was decreased about equally on both sides. In such instances, however, it is conceivable that at the times the experiments were performed both sides were growing at the same rate.

In some circumstances other substances which dissociate cellular proteins also increase growth. Of the agents which condition a dissociation, the following may at least initially accelerate growth: anesthetics (16, 39), carbon dioxide (57), disease (24), alternating currents (39), localized injury (57), and X-irradiation (15). Under certain conditions the parallelism between growth and the state of cellular proteins is striking. For example, PFEFFER (57), GASSNER (17), and HARTMANN (23) reported a greater growth on the positive side when plants were weakly stimulated by an electrical current and a greater growth on the negative side when more

strongly stimulated. These results parallel the effects that an electrical current has on the dissociation of cellular proteins as evidenced by viscosity changes. NORTHEN (51) noted that with short exposures to a current the decrease in structural viscosity was more extensive on the positive side than on the negative, whereas with longer exposures a decrease was observed on the negative side and an increase on the positive side. Hence it seems that the dissociations conditioned by a direct current, auxins, or other agents may at times condition an accelerated growth. When walls are sufficiently plastic, such increased growth may result from an increased protoplasmic swelling, a concept previously advanced by STRUGGER (72). However, auxins not only condition an increased swelling pressure, but—as HEYN (26) has demonstrated—they also condition an increase in the plasticity of cell walls. Such increased plasticity may or may not result through the intermediation (dissociation) of protoplasm.

Studies on the reactivity of cells to growth substances suggest that dissociation-association reactions of cellular proteins play a role in growth, although not necessarily a primary one in all instances. For example, BOYSEN-JENSEN (9) suggested that the positive phototropic curvatures of *Raphanus* hypocotyls were conditioned in part by a greater reduction in the reactivity of cells on the illuminated side than on the shaded side. The studies of WEBER (80), who noted that the viscosity of protoplasm was much higher in light than in darkness, suggest that the greater reactivity is conditioned by a greater dissociation of cellular proteins on the shaded side, on which side the swelling pressure would accordingly be greater.

The reactivity to unequal illumination may be modified by many substances. RINA and ARDUINO-JOLANDA (63) noted that plants watered with dilute solutions of copper sulphate, caffeine, quinine chlorhydrate, strychnine, ethyl alcohol, and nicotine showed a greater response to unilateral lighting than did plants watered with distilled water. RICHTER (62) observed that plants exposed to an atmosphere containing ether exhibited a greater heliotropic response than did control plants. In certain concentrations and with some exposures, caffeine (NORTHEN, unpublished observations), copper chloride (2), ethyl alcohol (25, 50), and ether (13, 50) condition a decrease in protoplasmic viscosity. One interpretation of the increased sensitivity may be as follows: The concentrations of the applied reagents were not high enough in the reacting cells to result in marked dissociation of the cellular proteins, although they were sufficient to condition the formation of labile proteins, which would be more readily dissociated by auxin (which accumulates on the shaded side) than native proteins.

According to this interpretation the sensitivity of a tissue is increased by agents which transmute native to labile proteins. This concept may also explain the data of HITCHCOCK and ZIMMERMAN (29, 30). They noted that auxin preparations which contained 0.01 per cent ethyl alcohol were more effective in inducing root

formation than similar preparations not containing alcohol; they found furthermore that greater than additive effects were obtained with mixtures composed of one active root-inducing substance and one or more substances of lower or no activity. In these instances the dilute alcohol and the substances of lower or no activity may have conditioned the formation of more sensitive labile proteins.

When growth is augmented the energy requirements are increased. Such requisites are generally fulfilled by an acceleration of respiration. BONNER (8) demonstrated that cell elongation is closely correlated with respiration. THIMANN (74) obtained evidence which associated the action of auxins with an oxidation reaction. Hence it seems that respiration is accelerated by the same structural alterations as those which augment swelling pressure.

When cellular proteins dissociate, disulphide linkages are broken, with resulting increase in the number of sulfhydryl (SH) groups. Such groups may actuate many respiratory enzymes. RAPKINE (60) noted that the enzyme which catalyzed the oxidation reduction between triose phosphate and pyruvate was active only when sulfhydryl groups were present. GILL and LEHMANN (18) quoted the work of QUASTEL and WHEATLEY and that of RUNNSTRÖM and SPERBER, which indicated that the respiration of yeast was increased by SH-glutathione and cysteine. Moreover, they noted that reducing compounds and agents which increased the content of SH groups in the medium augmented Robison ester formation.

Several investigators have found that dehydrogenases are actuated by SH groups (61, 32). That dehydrogenase activity is closely related to growth is evident from the investigation of COMMONER and THIMANN (12), who observed that dehydrogenase inhibitors restrained the growth of *Avena* coleoptiles. Furthermore, it is possible that during dissociation activators other than SH groups may be disengaged (such as ions), that substrate molecules or the enzymes themselves may be disunited and thereby increase the respiratory rate.

The quickened rate of respiration, conditioned by auxins or other dissociating agents, may in part be caused by an increase in the amount of respirable substrate as a result of polysaccharide hydrolysis. Carbohydrases as well as respiratory enzymes are actuated by sulfhydryl groups, formed when cellular proteins dissociate. MICHAELIS and RUNNSTRÖM (46) noted that the glucolytic enzyme of muscle extract was activated by SH groups. ITO (35) reported that the hydrolytic activity of α -amylase, β -amylase, and sucrase was accelerated by reduction and retarded by oxidation. In addition to the activation of carbohydrases by such reducing groups as sulfhydryl, the dissociation may result in the disengagement of enzymes from previous combination. KURSSANOV (37) states that enzymes are partitioned in the cell between the structural elements and the aqueous solution. In solution the enzymes hydrolyze and, when adsorbed, synthesize. Furthermore, he believes that changes in the physico-chemical conditions in the cell may modify

the ratio. When cellular proteins dissociate, carbohydrases may accumulate in the aqueous phase, thus conditioning hydrolysis. Hence it is likely that those structural alterations which condition increased swelling pressure and respiration also condition increased polysaccharide hydrolysis.

Growth substances often bring about the formation of roots, and such formation may be conditioned by the dissociation of cellular proteins. The appearances of cells and their activities are regulated primarily by the protoplasmic patterns within the cells. Hence, if cells which normally would not divide and develop into root cells are induced to do so, the protoplasmic patterns in the normal cells have been altered. Before a modified pattern can be produced the cellular proteins must be dissociated. Then the products of dissociation may be rearranged (reassociated) to form a dissimilar pattern, one that will function differently. This concept is in complete harmony with recent biochemical data. TISELIUS and HORSFALL (78) noted that after hemocyanins from different species were dissociated, the reassociated proteins differed from the original ones. PEDERSEN (56) determined the presence of an interaction between components in protein mixtures. Such interactions gave rise to new molecular types.

In the development of the abscission layer a series of protoplasmic changes occur. The dissociation of protoplasm by auxins will interrupt such orderly changes and thereby delay the formation of the layer.

Auxins, like many other substances, may stimulate, restrain, or kill, the end reaction depending upon concentration and time. THIMANN (74) has diagrammed the relationship of concentration to effect for stems, roots, and buds. In general, higher concentrations are required for retardation than for stimulation and still higher for death. The dissociation-association concept, outlined in the early part of this paper, adequately shows the interrelationship between death, retardation, and stimulation.

In general, actively growing tissues are more sensitive to potentially lethal agents than are dormant or nongrowing tissues. In actively growing tissues the cellular proteins are usually somewhat dissociated, whereas in dormant tissues the proteins are not. Dissociated proteins may be denatured more readily than native ones. LUNDGREN and WILLIAMS (43) found that dissociation and denaturation were closely related. When cellular proteins denature, death is near. To be sure, the sensitivity of tissues is closely related to water content. But such sensitivity is probably primarily conditioned by the state of the cellular proteins. That is, an increase in the water content of a cell may condition some dissociation with a concomitant increased sensitivity, respiration, carbohydrate hydrolysis, and swelling pressure. That dilution may condition a dissociating of cellular proteins is evident from the work of SVEDBERG (73), who noted that high dilution caused the dissociation of *in vitro* proteins.

Summary

1. The centrifuge method was used to determine protoplasmic viscosity in cortex cells (and in some instances in ray and pith cells) of bean petioles and stems which had been daubed with lanolin preparations of indole-3-acetic acid, indole-3 α -propionic acid, and α -naphthalene acetic acid.

2. Unilateral applications of the growth substances initially conditioned negative curvatures, in which instances the protoplasmic viscosity was lower in cells on the faster growing sides of the petioles and stems than in cells on the slower growing sides, and likewise lower than in control petioles or stems. When subsequent positive curvatures resulted, the viscosity was decreased about equally on the treated and untreated sides, except in the case of petioles treated with a paste containing 100 mg. of indole-3 α -propionic acid per gram of lanolin, in which instance the viscosity was lower on the untreated sides.

3. Applications of indole-3 α -propionic acid or α -naphthalene acetic acid to the tops of decapitated plants resulted in a decrease in the structural viscosity of protoplasm in cells below the cut surfaces.

4. In part at least, the decreases in structural viscosity were conditioned by dissociations of cellular proteins; that is, by the splitting of proteins into molecules one-half, one-fourth, one-eighth, etc., that of the original ones.

5. Biochemical and thermodynamical evidence is introduced which suggests that such dissociations condition increased protoplasmic swelling pressure, respiration, and polysaccharide hydrolysis, as well as altering development.

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LITERATURE CITED

1. ALEXANDER, T. R., Carbohydrates of bean plants after treatment with indole-3-acetic acid. *Plant Physiol.* 13:845-858. 1938.
2. ANGERER, C. A., The effect of salts of heavy metals on protoplasm. I. The action of cupric chloride on the viscosity of sea-urchin eggs. *Jour. Cell. Comp. Physiol.* 10:183-197. 1937.
3. ———, The effect of electric current on the relative viscosity of sea-urchin egg protoplasm. *Biol. Bull.* 77:399-406. 1939.
4. AUDUS, L. J., Mechanical stimulation and respiration rate in the cherry laurel. *New Phytol.* 34:386-402. 1935.
5. BEAL, J. M., Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. *BOT. GAZ.* 102:366-372. 1940.
6. BELLUCCI, B., Beiträge zur Frage des Angriffspunktes der Strahlen an der lebenden Materie. *Strahlentherapie* 65:547-568. 1939.
7. BLUM, J. L., Responses of sunflower stems to growth-promoting substances. *BOT. GAZ.* 102:737-748. 1941.
8. BONNER, JAMES, The action of the plant growth hormone. *Jour. Gen. Physiol.* 17:63-76. 1933.

9. BOYSEN-JENSEN, P., Growth regulators in the higher plants. *Ann. Rev. Biochem.* 7:513-528. 1938.
10. BROOKS, M. M., Comparative studies on respiration. XV. The effect of bile salts and saponin upon respiration. *Jour. Gen. Physiol.* 3:527-532. 1921.
11. CHAMBERS, L. A., The effect of intense sound waves on some biologically important chemical reactions. *Amer. Jour. Med. Sci.* 190:857. 1935.
12. COMMONER, B., and THIMANN, K. V., The relation between growth and respiration in the *Avena* coleoptile. *Jour. Gen. Physiol.* 24:279-296. 1941.
13. DAUGHERTY, K., The action of anesthetics on amoeba protoplasm. *Physiol. Zoöl.* 10:473-483. 1937.
14. DELAGE, Y., Experimental parthenogenesis by electric charges. *Compt. Rend.* 147:553-557. 1909. (*Chem. Abst.* 3:193. 1909.)
15. DIETZ, T. J., Volume increase of bacteria from X-ray irradiation. *Radiology* 24:31-38. 1935.
16. GAIN, E., Effets de l'anesthésie sur la croissance d'*Allium*: observation du choc anesthésique. *Compt. Rend. Soc. Biol.* 93:763-764. 1925.
17. GASSNER, G., Über die Untersuchungen von J. Zeidler zur Frage des Galvanotropismus der Wurzeln. *Ber. deutsch. Bot. Ges.* 41:17-19. 1923.
18. GILL, P. M., and LEHMANN, H., Some factors influencing the formation of Robison ester from glycogen and inorganic phosphate in muscle extract. *Biochem. Jour.* 33:1151-1170. 1939.
19. GODWIN, H., The effect of handling on respiration of cherry laurel leaves. *New Phytol.* 34:403-406. 1935.
20. GREENLEAF, W. H., Induction of polyploidy and sterility in amphidiploids by hetero-auxin treatment. *Amer. Jour. Bot.* 26:673. 1939.
21. HAAS, A. R. C., Effect of anesthetics upon respiration. *BOT. GAZ.* 67:377-404. 1919.
22. HAND, D. B., Molecular weight and association of the enzyme urease. *Jour. Amer. Chem. Soc.* 61:3180-3183. 1939.
23. HARTMANN, H., Reaktionen von Koleoptilen und Wurzeln im Elektrischen Feld. *Beitr. Biol. Pflanzen (Cohn)* 19:287-333. 1932.
24. HEALD, F. D., *Manual of plant diseases.* New York. 1926.
25. HEILBRUNN, L. V., *An outline of general physiology.* Philadelphia. 1937.
26. HEYN, A. N. J., The physiology of cell elongation. *Bot. Rev.* 6:515-574. 1940.
27. HINDERER, T., The alteration of hereditary tendencies under the action of carbon dioxide. *Arch. Entwickl. Organ.* 38:187-209; 364-401. 1914. (*Chem. Abst.* 8:1310. 1914.)
28. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Use of physiological responses for determining absorption and transport of synthetic growth substances added to soil. *Contr. Boyce Thompson Inst.* 7:447-476. 1935.
29. ———, The use of green tissue test objects for determining the physiological activity of growth substances. *Contr. Boyce Thompson Inst.* 9:463-518. 1938.
30. ———, Effect obtained with mixtures of root inducing and other substances. *Contr. Boyce Thompson Inst.* 11:143-160. 1940.
31. HOPKINS, E. F., Variation in sugar content in potato tubers caused by wounding and its possible relation to respiration. *BOT. GAZ.* 84:75-88. 1927.
32. HOPKINS, F. G., MORGAN, E. J., and LUTWAK-MANN, C., The influence of thiol groups in the activity of dehydrogenases. II. *Biochem. Jour.* 32:1829-1847. 1938.
33. HOWARD, W. L., Physiological changes accompanying breaking of the rest period. 5th report. *Missouri Agr. Exp. Sta. Res. Bull.* 21. 1915.

34. IRWIN, M., Comparative studies on respiration. VI. Increased production of carbon dioxide accompanied by decrease of acidity. Jour. Gen. Physiol. 1:399-403. 1919.
35. ITO, R., Effects of reduction and oxidation on the reversible action of α -amylase, β -amylase, amylosynthase, sucrase, and urease. Sitzber. freien arztl. Ver. Med. Fakultät Kaiserl. Kyūsyū Univ. Hukuoka (266 Sitzung 16 Dez., 1938); Hukuoka Acta Med. 32:88. 1939. (Chem. Abst. 35:5917. 1941.)
36. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. 98:370-420. 1936.
37. KURSSANOV, A. L., Untersuchung enzymatischer prozesse in der Lebenden Pflanze. Advances Enzymol. 1:329-370. 1941.
38. KÚTHY, A. VON, The effect of surface-active substances on gelatins. II. Gel-sol transformation. Biochem. Zeitschr. 244:331-336. 1932.
39. LAIBACH, F., Interferometrische Untersuchungen an Pflanzen. II. Die Verwendbarkeit des Interferometers in der Pflanzenphysiologie. Jahrb. wiss. Bot. 76:218-282. 1932.
40. LEBEDEV, A., Concerning the action of the alternating current upon the hydrolyzing characteristic of diastase and mineral acids. Biochem. Zeitschr. 9:392-402. 1909. (Chem. Abst. 3:188. 1909.)
41. LLOYD, D. J., Protein structure and water absorption. Jour. Phys. Chem. 42:1-10. 1938.
42. LOEB, J., On the fertilization and cytolytic effect of soap. Proc. Soc. Exp. Biol. Med. 6: advance sheets. 1909.
43. LUNDGREN, H. P., and WILLIAMS, J. W., Ultracentrifugal analysis and stability in protein systems. Jour. Phys. Chem. 43:989-1002. 1939.
44. MATHEWS, A. P., Division of unfertilized eggs. Amer. Jour. Physiol. 4:343-347. 1900.
45. MCCLENDON, J. F., On artificial parthenogenesis of the sea-urchin egg. Science 30:454-455. 1910.
46. MICHAELIS, L., and RUNNSTRÖM, J., Inactivation and regeneration of the glucolytic enzyme system of muscle extract. Proc. Soc. Exp. Biol. Med. 32:343-349. 1934.
47. MITCHELL, J. W., Effect of naphthalene acetic acid and naphthalene acetamide on some nitrogenous and carbohydrate constituents of bean plants. BOT. GAZ. 101:688-699. 1940.
48. MITCHELL, J. W., KRAUS, E. J., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves following spraying with alpha-naphthalene acetic acid emulsion. BOT. GAZ. 102: 97-104. 1940.
49. MUIR, R. M., Effect of bile salts and oleates on the structural viscosity of protoplasm. BOT. GAZ. 102:357-365. 1940.
50. NORTEN, H. T., Protoplasmic structure in *Spirogyra*. III. Effects of anesthetics on protoplasmic elasticity. BOT. GAZ. 100:238-244. 1938.
51. ———, Studies on the protoplasmic nature of stimulation and anesthesia. II. Plant Physiol. 15:645-660. 1940.
52. ———, Effects of protein dissociating agents on the structural viscosity of protoplasm. Biodynamica 3:10-27. 1940.
53. NORTEN, H. T., and MACVICAR, R., Effect of X-rays on the structural viscosity of protoplasm. Biodynamica 3:28-32. 1940.
54. NORTEN, H. T., and NORTEN, R. T., Studies of protoplasmic structure in *Spirogyra*. II. Alterations of protoplasmic elasticity. Protoplasma 31:9-19. 1938.
55. NORTHROP, J. H., The swelling of isoelectric gelatin in water. Jour. Gen. Physiol. 10:893-904. 1927.
56. PEDERSEN, K. O., Ultracentrifugal studies on protein mixtures. Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim. 22:427-433. 1938.

57. PFEFFER, W., *Physiology of plants* (Engl. trans. by A. J. EWART). Oxford. 1905.
58. PRATT, R., Influence of indole-3-acetic acid on the respiration and growth of intact wheat seedlings. *Amer. Jour. Bot.* 25:389-392. 1938.
59. ———, Respiration of wheat infected with powdery mildew. *Science* n.s. 88:62-63. 1939.
60. RAPKINE, L., Sulfhydryl groups and enzymic oxidation-reduction. *Biochem. Jour.* 32:1729-1739. 1938.
61. RAPKINE, L., and TRPINAC, P., Role of sulfhydryl groups of proteins in the activity of dehydrogenases. *Compt. Rend. Soc. Biol.* 130:1516-1518. 1939.
62. RICHTER, O., Increase in the heliotropic sensitiveness of seedlings due to narcotics. *Sitzb. Akad. Wiss. Wien, Math-Naturw.* 121:1183-1228. 1914. (Chem. Abst. 8:957. 1914.)
63. RINA, G., and ARDUINO-JOLANDA, R., Contributo allo studio dell'eliotropismo nelle piante. L'azione di diverse sostanze eccitanti sopra di esso. *Natura* (Milano) 18:1-27. 1927. (Biol. Abst. 3:830. 1929.)
64. SCHMIDT, C. L. A., *The chemistry of the amino acids and proteins*. Baltimore. 1938.
65. SHARP, L. W., *Introduction to cytology*. New York. 1934.
66. SHULL, C. A., and MITCHELL, J. W., Stimulative effects of X-rays on plant growth. *Plant Physiol.* 8:287-296. 1933.
67. SNOW, R., Activation of cambial activity by pure hormones. *New Phytol.* 34:347-360. 1935.
68. SÖDING, H., Über das Wachstum der Infloreszenzschäfte. *Jahrb. wiss. Bot.* 77:627-656. 1933.
69. SOKOLOV, I. I., Structure and physicochemical properties of "disaggregated" gelatin. *Colloid Jour. (U.S.S.R.)* 4:533-549. 1938. (Chem. Abst. 33:6120. 1939.)
70. SPRENSSEN, S. P. L., The constitution of soluble proteins (reversibly dissociable component systems). *Compt. Rend. Trav. Lab. Carlsberg* 18:1-124. 1930.
71. STEINHARDT, J., Total dissociation of horse hemoglobin. *Nature* 138:800-801. 1936.
72. STRUGGER, S., Beiträge zur Physiologie des Wachstums. I. Zur protoplasma-physiologischen Kausalanalyse des Streckungswachstums. *Jahrb. wiss. Bot.* 79:406-471. 1934.
73. SVEDBERG, T., Protein molecules. *Chem. Rev.* 20:81-98. 1937.
74. THIMANN, K. V., Hormones and the analysis of growth. *Plant Physiol.* 13:437-449. 1938.
75. THIMANN, K. V., and BONNER, J., Plant growth hormones. *Physiol. Rev.* 18:524-553. 1938.
76. THORNTON, N. C., Carbon dioxide storage. V. Breaking the dormancy of potato tubers. *Contr. Boyce Thompson Inst.* 5:470-480. 1933.
77. ———, Carbon dioxide storage. VIII. Chemical changes in potato tubers resulting from exposure to carbon dioxide. *Contr. Boyce Thompson Inst.* 7:113-118. 1935.
78. TISELIUS, A., and HORSFALL, F. L., Mixed molecules of hemocyanins from two different species. *Jour. Exp. Med.* 69:83-101. 1939.
79. TURFITT, G. E., A new method for the determination of phytohormone activity. *Biochem. Jour.* 35:237-244. 1941.
80. WEBER, F., Plasmolysezeit und Lichtwirkung. *Protoplasma* 7:256-258. 1929.
81. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Experiments with vapors and solutions of growth substances. *Contr. Boyce Thompson Inst.* 10:481-508. 1939.

RESISTANCE OF CUCUMBER SEEDLINGS TO DAMPING-OFF AS RELATED TO AGE, SEASON OF YEAR, AND LEVEL OF NITROGEN NUTRITION¹

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(WITH TWENTY FIGURES)

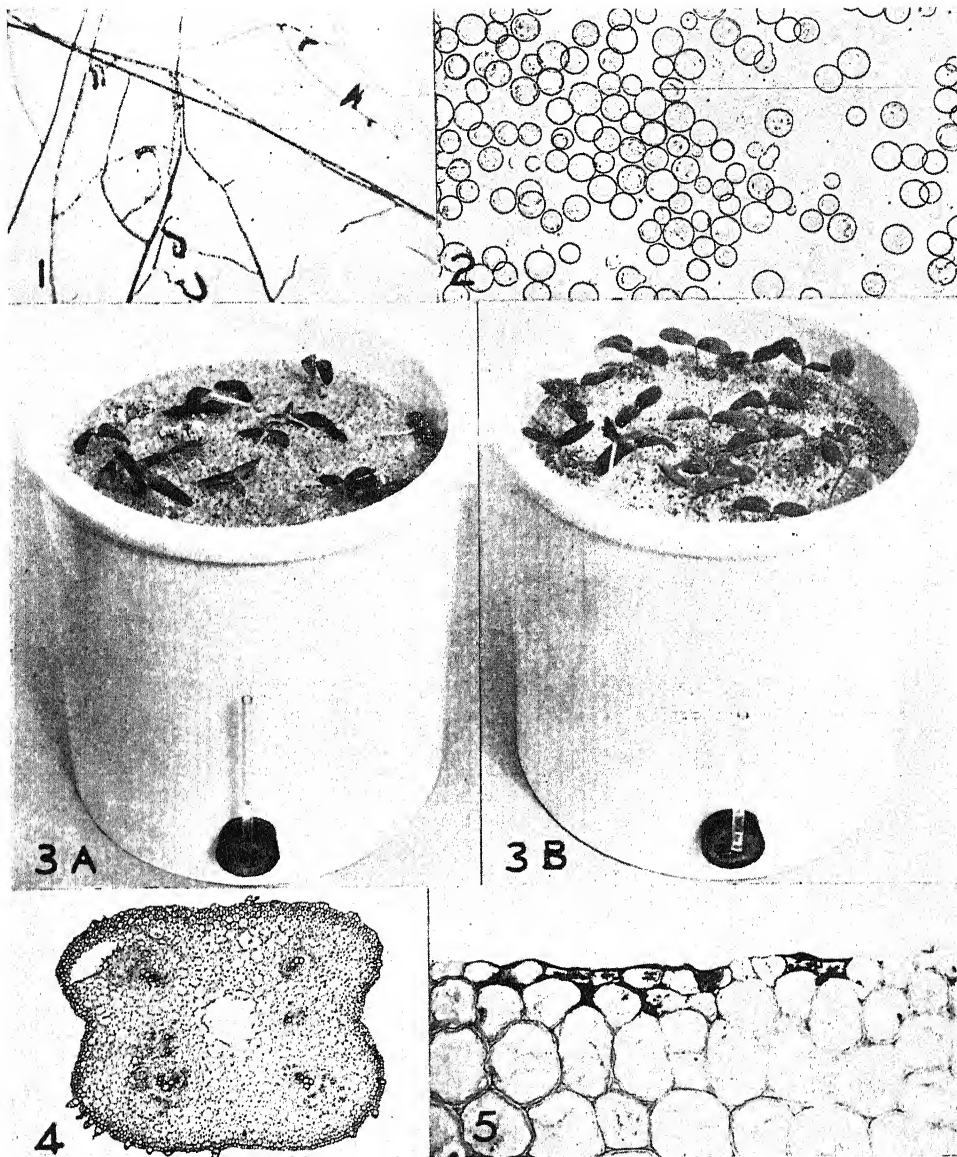
Introduction

This paper presents the results of an investigation on the manner in which cucumber seedlings show different degrees of resistance to damping-off after being inoculated with a form of *Pythium*. The lots of seedlings tested differed with regard to the level of nitrogen in the nutrient solutions with which they were supplied and with regard to age and season of the year in which they were grown. Inoculations of seedlings with the fungus culture were made at frequent intervals from May to July, 1937, and from November to April, 1938. Structural modifications of tissues of the seedlings associated with the relative resistance to the infection were studied.

Damping-off diseases are generally considered more likely to occur in seedlings than in older plants, and resistance to infection by the causal fungus organisms increases as seedlings grow older. The fungi causing damping-off have an exceedingly wide host range and can grow parasitically or saprophytically. Because of the wide range of environmental conditions under which these organisms may develop, a number of environmental factors have been associated with resistance or susceptibility to infection. It has been noted that high temperature, high humidity (25), high soil moisture (4), and deficiency in the light supply (1) all favor development of the diseases. The generalization has been made that seedlings in a "weakened" condition are more susceptible than are more vigorously growing ones (8), although this suggestion does not throw much light on the nature of the resistance.

Regarding the influence of the mineral nutrition of the host on susceptibility to infection, LAURENT (according to JONES, 12) concluded that field use of nitrogenous fertilizers predisposed foliage and tubers of potato to infection by *Phytophthora* but COOK (6) found that tomato plants grown with low nitrate supply were unusually susceptible to infection by *Fusarium lycopersici*.

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FIGS. 1-5.—Fig. 1, allantoid bodies *Pythium splendens*. Fig. 2, conidia in Mehrlich's liquid medium. Fig. 3, cucumber seedling inoculated at 6 days of age: A, with deficient nitrogen; B, with adequate nitrogen. Fig. 4, transection of hypocotyl of a 6-day healthy seedling. Fig. 5, transection of scar formation; hypocotyl of resistant seedling.

The resistance of plants to infection by pathogenic fungi has often been ascribed to some morphological feature of the plant tissue. HARTLEY (9) thought that the spread of *Pythium debaryanum* in conifer tissues was hindered by thick cell walls, and HEALD (10) found an inverse correlation between the thickness of parenchyma walls and the spread of *Phytophthora infestans* in potato tubers. In some host plants the progress of an infecting fungus may be greatly retarded or stopped by special growth reactions of neighboring cells. BRAUN (3) found that the resistance to the spread of *Pythium complectens* in geranium cuttings was associated with the formation of a corky layer in advance of the fungus. TISDALE (23) observed a similar walling-off in *Linum* when invaded by *Fusarium*, but ULLSTRUP (24) found no such barrier to the advance of *Fusarium* in China aster wilt. CONANT (5) reported a close correlation between the resistance of tobacco seedlings to infection by *Thielavia basicola* and cork formation in the tissues underlying the lesions.

Experimental methods

CULTURE OF HOST PLANTS.—Cucumber (*Cucumis sativus* Linn.) was chosen for this study because this species is very susceptible to infection by Pythiaceae

TABLE 1
COMPOSITION OF NUTRIENT SOLUTIONS EXPRESSED AS
VOLUME MOLECULAR CONCENTRATIONS OF SALTS*

SOLUTION	KH ₂ PO ₄	CaCl ₂	Ca(NO ₃) ₂	MgSO ₄	(NH ₄) ₂ SO ₄
Complete.....	0.00105	0.00365	0.00355	0.0007
Without nitrogen.....	0.00105	0.00365	0.00355

* Iron, boron, and manganese used in each solution in concentrations of 1, $\frac{1}{2}$, and $\frac{1}{2}$ p.p.m., respectively.

fungi (7, 22) and because the seedlings are readily handled in experimentation of this kind. Cucumber seeds of the variety Improved Long Green were surface treated for a few minutes with 0.1 per cent aqueous solution of mercuric chloride, after which they were rinsed with sterile water and allowed to germinate between moist sheets of sterile blotting paper in covered glass crystallizing dishes at 24° C. After a germination period of 30 hours, apparently healthy seedlings with radicles protruding about 1 mm. were selected and planted 1 cm. deep in quartz sand held in 2-gallon glazed crocks (fig. 3) with bottom drainage.

Each culture was supplied with about 1 liter of nutrient solution per day, according to the constant drip procedure of SHIVE and STAHL (20). Some cultures received a complete nutrient solution; others received a solution lacking nitrogen. These solutions were modifications of JONES and SHIVE's (13) four-salt solution T₁R₁C₅, except that the osmotic concentration was equivalent to about 0.5 atmos-

phere. The composition of these solutions is given in table 1. The seedlings supplied with the complete solution had an adequate supply of nitrogen, whereas in the other treatment the seedlings received no nitrogen except that which was derived from the seeds or present as unavoidable impurities introduced with the use of analytically pure nutrient salts of the grade Baker's Analyzed.

TABLE 2

INCIDENCE OF INFECTION IN SEEDLINGS GROWN WITH ADEQUATE AND WITH INADEQUATE NITROGEN SUPPLY AND INOCULATED ON HYPOCOTYL WITH PYTHIUM AT VARIOUS AGES AND AT VARIOUS TIMES OF YEAR

DATE		AGE (DAYS) AT START OF INOCULATION (AND NO. OF DAYS IN IN- OCULATION PERIOD)	INCIDENCE OF INFECTION (KILLED OR SCARRED)					
CULTURE STARTED	INOCU- LATION STARTED		ADEQUATE NITROGEN			DEFICIENT NITROGEN		
			No. IN- OCULATED	KILLED (%)	SCARRED (%)	No. IN- OCULATED	KILLED (%)	SCARRED (%)
5/5/37.....	5/18/37	13 (7)	15	0	27	18	0	61
5/12.....	5/18	6 (7)	18	0	61	19	95	5
6/9.....	6/22	13 (4)	20	0	10	20	0	30
6/9.....	6/22	13 (4)*	10	0	100	10	10	90
6/16.....	6/24	8 (4)	20	0	25	20	0	40
6/22.....	6/28	6 (2)	20	100	0	20	100	0
6/30.....	7/8	8 (2)	10	0	100	9	0	100
10/13.....	11/2	20 (4)	16	0	25	16	0	38
10/27.....	11/2	6 (4)	20	100	0	18	100	0
10/27.....	11/13	17 (4)	20	50	30
11/3.....	11/13	10 (4)	20	100	0	20	100	0
11/24.....	12/7	13 (4)	20	100	0
12/1.....	12/7	6 (4)	20	100	0
12/1.....	12/14	13 (4)	20	95	5
12/16.....	1/5/38	20 (3)	31	100	0
12/16.....	1/5	20 (3)	15	100	0
12/16.....	1/13	28 (2)	16	100	0
2/18/38.....	3/19	29 (2)	10	90	10	10	70	30
3/6.....	3/19	13 (2)	19	100	0	20	100	0
3/11.....	4/10	28 (2)	9	11	89
3/11.....	4/10	28 (2)	5	40	60
3/29.....	4/14	16 (2)	10	33	67	9	22	78
3/29.....	4/14	16 (2)	10	50	50	9	67	33

* Data in heavy type are for tests in which inoculation was applied to fresh wound.

The dates on which the sand cultures were started and the duration of their treatments before the seedlings were inoculated are given in table 2. Each culture was continued through the inoculation period and usually for a few days thereafter, to permit any evidences of infection to become unmistakably apparent. The culture crocks stood on a greenhouse bench during the experiments, except during the winter months when they were inclosed in a special chamber glazed with trans-

parent cellulose acetate film. The temperature in this chamber was kept above 20° C. by thermostatically controlled electric heating cable. The day length and average light intensity varied according to the time of year and variations in diurnal cloudiness.

CULTURE OF FUNGUS.—The *Pythium* used was isolated from lettuce seedlings showing the damping-off disease. It is closely similar to *P. splendens* (3) which grows over a temperature range of 11°–37° C., with an optimum temperature of about 30° C. The H-ion requirements of *P. splendens* are not reported, but the closely related *P. debaryanum* has been found to grow well from pH 5 to 7 (2). The fungus of the present study grew readily on potato agar, rice mush, MEHR-LICH's agar, or in MEHR-LICH's liquid (14). The inoculum was grown on potato agar or rice mush. The *Pythium* showed strong aerial growth on rice mush, but its aerial growth on 1.5 per cent agar media was much less vigorous. On stiff agar, and when in contact with glass, the laterals were often allantoid in shape (fig. 1). The hyphal diameter was usually about 5.8 μ in the region where the conidia were borne, varying elsewhere from 1.9 to 8.2 μ . Neither zoospores nor oögonia were seen. The nearly spherical conidia (fig. 2) were profuse, usually terminal, with a diameter of 21.7–48.0 μ , usually about 37 μ .

INOCULATION OF SEEDLINGS.—The technique followed for inoculation was essentially like that described by RATHBUN (18). Similar sized pieces of agar or rice mush about 5 mm. in diameter were cut from a plate culture of the fungus, and one of these was applied against one side of the hypocotyl of each of the seedlings to be inoculated, being left in position until the end of the test in each instance. In most of the tests the inoculum was applied to the hypocotyl about 1 cm. above the sand level and when necessary was supported in this position with a tiny cardboard platform. This technique was necessary since the fungus sometimes grew downward through the sand to the seedling roots when the inoculum rested on the sand surface.

In some instances (as indicated by the data in heavy type in table 2) the inoculation was made by slightly scratching the hypocotyl on one side with a needle and immediately applying the inoculum against this lesion. It was thought that this procedure might facilitate primary infection after inoculation, but the results were not very different from those secured without preliminary wounding. After inoculation, the cultures were covered with bell jars, thus providing humidity to keep the inoculum from drying out.

From ten to twenty seedlings were usually inoculated in each test, and the number subsequently killed by the disease was computed as a percentage of the number inoculated. When the seedlings were not killed, primary infection occurred in many cases but failed to develop beyond a small region close to the place of inoculation. In such cases the primary lesion healed, leaving a notable in-

fection scar which was much more pronounced than the slight scratch resulting from the preliminary wounding. For each test the number of seedlings thus scarred by incipient but abated infection was also computed as a percentage of the number of seedlings inoculated. These two percentages are taken as indices of the degree of susceptibility of the seedlings in each test (table 2). For example, of the fifteen seedlings grown with adequate nitrogen supply that were inoculated on May 18, 1937, none were killed but 27 per cent showed infection scars; while of the eighteen seedlings grown with inadequate nitrogen supply and inoculated at that time, none were killed but 61 per cent showed the scars.

HISTOLOGICAL EXAMINATION OF SEEDLINGS.—Fresh samples of tissue were killed and fixed in Allen's PFA₃ solution (15) and subjected to reduced pressure. Dehydration was carried out with 50 per cent ethyl alcohol and then with butyl alcohol (11). Paraffin sections were cut 8–10 μ thick. For staining, a combination of safranin and fast green was found useful in the study of seedling tissues generally, but a combination of crystal violet and gold orange was especially valuable in showing sharp differentiation between healthy and diseased tissue, while a combination of magdala red and fast green served for contrast between host and parasite.

Among the microchemical tests the following were used. For starch: iodine-potassium-iodide; for cellulose: (a) iodine-sulphuric acid and (b) chlor-iodide of zinc; for lignin: (a) phloroglucin, (b) iodine-sulphuric acid, and (c) potassium permanganate;² for cuticle and cuticularized walls: Sudan IV; for cuticularized walls only: iodine-sulphuric acid. Except for the test with potassium permanganate, these reagents were prepared and used according to the directions given by RAWLINS (19).

Results

The results of infection of the seedlings of various ages after inoculation are recorded in table 2. Seedlings which succumbed to infection were counted as highly susceptible and were designated killed. Seedlings which after inoculation formed slight but visible scars on the hypocotyl at the point of inoculation were designated scarred. These seedlings, however, were considered resistant to complete infection by the organism if further development of the disease did not occur. Inoculated seedlings counted as completely resistant to infection generally showed very slight scars which, although not visible, became evident upon histological examination. This is shown in figure 5 by the darkened cell walls in the epidermal region of transverse section of the hypocotyl.

From the data presented in table 2 it appears that the relative resistance to infection of seedlings of a given age varies with the season of the year. During the winter months seedlings were susceptible at ages at which during the summer months they were found to be resistant. Thus on May 18 and on June 22, 13-day-

² ECKERSON, SOPHIA H., Microchemistry (mimeographed).

old seedlings supplied with adequate nitrogen were entirely resistant to complete infection; whereas on December 14, seedlings of the same age with the same nutrient treatment were 95 per cent susceptible and were killed. For the interval of tests from November 2 until March 19, high susceptibility prevailed for seedlings adequately supplied with nitrogen at ages of 6–20 days. The inoculations of January 13 showed complete susceptibility, amounting to death of 100 per cent of the seedlings as old as 28 days. On April 10, 28-day-old seedlings adequately supplied with nitrogen were only 11 per cent susceptible to infection.

With regard to the nitrogen-deficient seedlings, the meager evidence seems to indicate somewhat similar seasonal trends in the relative susceptibility. During the period from about November 2 to about March 19 these seedlings showed high susceptibility, which, however, decreased during the early summer period of May and June.

When the nitrogen level is considered, seedlings adequately supplied with nitrogen were in most cases more resistant to infection than were those with a deficient nitrogen supply. In those tests where death of the seedling did not occur, the greater initial susceptibility of the low nitrogen seedlings is indicated by the consistently greater percentage of scarred seedlings (table 2). On May 18, for example, 95 per cent of the 6-day-old low nitrogen seedlings were killed, whereas none adequately supplied with nitrogen died. Figure 3*A* shows seedlings with a deficient nitrogen supply succumbing as a result of susceptibility. Figure 3*B* shows the resistance of seedlings adequately supplied with nitrogen.

Although this evidence is not extensive, it may warrant the conclusions: (*a*) that young seedlings were generally less susceptible to infection when grown with adequate nitrogen supply than when grown at a low level of nitrogen nutrition; (*b*) that seedlings of any given age were less susceptible to infection in early summer than in winter; (*c*) that the degree of susceptibility of young seedlings appeared generally to decrease with increasing age; and (*d*) that a high degree of susceptibility apparently persisted to a greater age in winter than in early summer.

That seedlings of similar age were much less resistant in early summer than in winter may safely be attributed to seasonal differences in their metabolism, for it is not reasonable to suppose that the composition of the stored seeds became effectively altered in a few months. Progressive physiological changes taking place with advancing age in the direction of what may be called maturation proceeded more rapidly in early summer than in winter, accounting for the fact that seedlings remained susceptible to infection for a longer time during the winter than during the summer season. Except for the influence of fluctuating cloudiness, the two seasonal factors most directly associated in these experiments with seasonal changes were the changes in length of photoperiod and in light intensity. Both factors affect the assimilation of carbon and thereby the available energy for all

endothermic metabolic plant processes. It was not determined which factor was the more important one concerned with seasonal change in resistance. It might readily be expected that the use of artificial light of suitable quality, intensity, and duration might have rendered the winter-grown seedlings just as resistant as the early summer-grown ones.

Histological examination of the hypocotyls of inoculated seedlings that had survived, and particularly of those which had been wounded at the point of inoculation, indicated that resistance to infection was seldom or never due to failure of the fungus to penetrate the epidermis. In the cases examined there was always some penetration and spread of the fungus from the place of entrance. The spread of the mycelium within the tissue was often very slight (fig. 5), but it was more extensive when clearly apparent infection scars were formed. In instances where seedlings survived, it was clear that some change in the host tissue near the region of primary penetration had occurred, and that such changes were associated with and perhaps the cause of the stopping of the further advance of the parasite.

The pH values of the hypocotyl parenchyma of the seedlings grown with adequate nitrogen supply (pH 5.6–5.8) were found to be generally slightly lower than for those with inadequate nitrogen (pH 5.8–6.0), but these pH values lie within the range for good growth of this *Pythium*. No difference was found between the pH values of hypocotyl parenchyma from very susceptible and from highly resistant seedlings.

Microchemical tests showed accumulations of considerable starch in the hypocotyls of the low nitrogen seedlings but no such starch accumulation in similar tissues of those adequately supplied with nitrogen. The mere presence of starch was not necessarily directly correlated with susceptibility, however, because low nitrogen seedlings were found to contain starch even after they had gained a certain degree of resistance with increase in age. The accumulation of starch in the low nitrogen seedlings was to be expected, since this phenomenon is a common occurrence in many other species of plants, accompanying a low rate of nitrogen assimilation (16, 17).

Hypocotyls of highly resistant seedlings were killed by freezing, heating, or treatment with strong alcohol, and after inoculation were found to support vigorous growth of the fungus used in this experiment. Hyphae were found to have penetrated throughout the dead host tissue, thus suggesting that resistance to infection ceases when the hypocotyl is killed and that resistance depends upon the activity of living cells—or at least upon features no longer effective after their death.

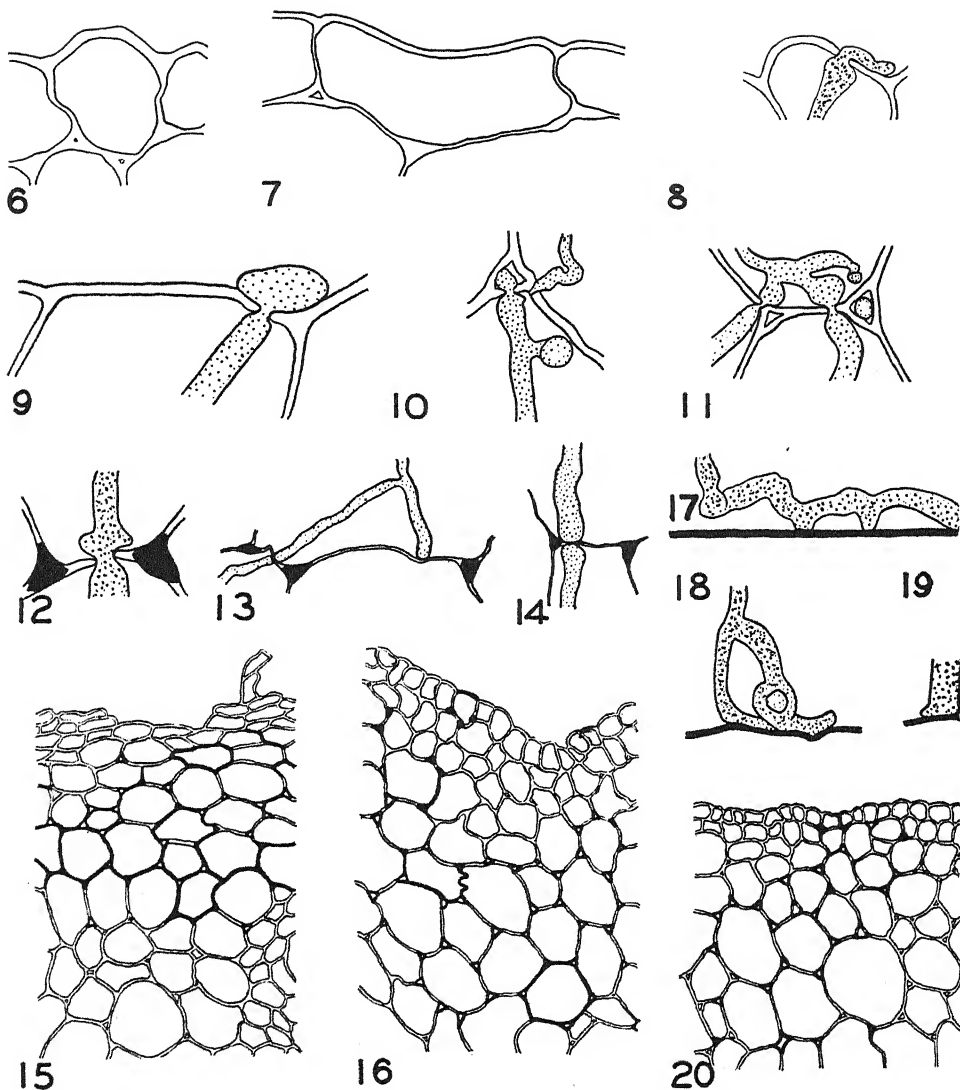
By means of transverse sections cut 1 cm. above the sand level, uninoculated hypocotyls of healthy young seedlings—like those found to be highly susceptible to infection—were compared with uninoculated hypocotyls of older seedlings,

such as those found to be highly resistant. Except for size they were very similar, and every type of tissue present in the older hypocotyls was already present in the younger ones (fig. 4). Average wall thickness in the fundamental tissues was the same in both cases—about $0.5\ \mu$.

The thickness of the outer epidermal walls, including the cuticle, had apparently decreased slightly with increasing age of the seedlings; in the younger seedlings these walls were about $2.0\ \mu$ thick (fig. 6), whereas they were only about $1.5\ \mu$ thick in the older seedlings (fig. 7), perhaps owing to stretching without corresponding thickening. The cuticle was of the same thickness in both cases—about $0.5\ \mu$. The outer cell walls themselves were composed mainly of cellulose but were slightly cuticularized. The cuticle showed no cellulose reaction. Figures 8 and 9 illustrate the penetration of the fungus into epidermal cells of the hypocotyl of a susceptible and of a resistant seedling, respectively. It therefore appeared that the high degree of resistance which characterized the older hypocotyls in contrast to the younger ones was not due to any maturation change in either cuticle or epidermal walls. In resistant hypocotyls, as has been said, hyphae from the inoculum penetrated somewhat beyond the epidermis (figs. 10, 11), even when no obvious scar resulted, and they were found to have advanced through several cell layers in cases where scars were to be found; but in such cases the spread of the initial infection soon ceased. In highly susceptible hypocotyls the mycelium spread rapidly through the fundamental tissue of the host.

Further examination of the diseased seedling tissue indicated the probable means by which resistance to infection occurred. When microchemical tests for lignin were applied to transverse sections cut through the infection scars, lignification was shown to be much more prevalent beneath and around the tissue of the lesion than elsewhere, suggesting that the spread of the fungus might in these cases have been limited by a barrier of lignified cells. In no case were hyphae seen to have penetrated through lignified regions of cell walls, although they did through nonlignified portions (figs. 12-14). It was not unusual to find hyphae growing in contact with lignified regions of walls as if unable to penetrate them (figs. 17-19). The importance of cork formation in the tissues beneath a lesion in tobacco seedlings has been emphasized by CONANT (5), who found that such tissue changes occurred more rapidly in varieties highly resistant than in others highly susceptible to infection by *Thielavia basicola*.

The prevalence of lignification in the region adjacent to the scar in the hypocotyl of a resistant seedling grown with adequate nitrogen is shown in figure 15. This extensive lignification, however, indicated in the figure by the blackened portions of the walls, was not accompanied by any thickening of the walls so affected. Figure 16 shows the infected area of the hypocotyl of a young high nitrogen seedling which was in an advanced stage of infection. Lignification of walls was



FIGS. 6-20.—Fig. 6, epidermal cell of 6-day seedling. Fig. 7, of 17-day seedling. Fig. 8, fungus penetration of epidermal cell; hypocotyl of 6-day susceptible seedling. Fig. 9, fungus penetration of epidermal cell of hypocotyl of 30-day resistant seedling. Figs. 10, 11, fungus penetration in parenchymatous tissue. Figs. 12-14, same of unligified portions of parenchymatous tissue; lignification shown as solid black. Fig. 15, transection of hypocotyl of 30-day resistant seedling grown with adequate nitrogen after wounding and inoculation. Fig. 16, hypocotyl of 7-day seedling susceptible after inoculation; adequate nitrogen. Figs. 17-19, fungus hyphae in contact with lignified cell walls. Fig. 20, hypocotyl of diseased 30-day low nitrogen seedling.

incomplete. Likewise lignification of cell walls in the tissues of low nitrogen seedlings was not so rapid or so complete in response to inoculation as that occurring in the tissues of resistant seedlings adequately supplied with nitrogen. Figure 20 is a transverse section of the hypocotyl of a 30-day-old, low nitrogen seedling susceptible to infection and shows incomplete lignification of cell walls and absence of a continuous lignified barrier. Fungus hyphae, although not shown in this figure, were seen throughout the tissue of this region.

Many observations of this sort led to the conclusion that the resistance which occurred in the hypocotyls of the seedlings was associated with the formation of lignin barriers and that such barriers were of a nature effectually to prevent further spread of the parasite through the host tissues. Whether such lignification barriers constituted the principal cause of resistance to the invasion through the tissues, or were formed concomitantly as a result of other changes in metabolism which rendered the seedlings resistant, cannot be definitely stated. The known fact that lignin is markedly resistant to enzymatic digestion, however, lends support to the belief that the mere development of a well-developed barrier of lignin would be sufficient effectually to limit the rapid spread of this organism. The formation of such lignin barriers in the tissues appeared to be favored by adequate nitrogen nutrition of the seedlings.

No such complete or nearly complete lignification of cell walls of the fundamental parenchyma was seen in sections from uninoculated hypocotyls that had not been wounded; and sections from badly infected hypocotyls, where spread of the fungus had not been restricted, showed little lignification and no such barriers. But sections of hypocotyls wounded with a sterile needle and not inoculated showed pronounced lignification barriers similar to those resulting from limited infection.

It appears that the capacity for this lignification response to fungus infection, a response apparently protective in its results, is related to physiological maturation of the tissues involved and that the hypocotyl tissues at the region of inoculation developed that physiological capacity more rapidly and sooner when the plants were grown with an adequate nitrogen supply and with long photoperiods of high light intensity levels than at a low nitrogen level or with short photoperiods of low light intensity levels.

Discussion

As indicated in this study, if resistance to infection is associated with and perhaps significantly dependent upon rapid lignification of cell walls surrounding the region of inoculation, it becomes of interest to consider how the factors found to be associated with relative resistance (level of nitrogen nutrition, age of seedlings, and season of year) are related to the rate of cell-wall lignification.

The deposition in cell walls of lignin, a carbohydrate derivative, is dependent

upon the presence of an adequate tissue content of available carbohydrate (21). During the days of low light intensity in the winter season, carbohydrate synthesis is at a relatively low plane. The rate of lignification was found to be low, and likewise the susceptibility to infection during this season of the year was found to be greater than during the late spring or early summer.

With regard to the increasing resistance to infection with increasing age of seedlings, it may be emphasized that—beginning with germination and during early growth—there is at first a decrease in the level of carbohydrate available for growth. This proceeds until, with the increasing efficiency of the photosynthetic mechanism, the seedling becomes independent of stored carbohydrate. This change takes place more rapidly with adequate than with a low light intensity and may account for the increase in resistance with advancing age and the differences in the ages at which the seedlings become resistant at different seasons of the year. In view of this explanation of resistance, it might be questioned why the seedlings grown with a low plane of nitrogen nutrition showed greater susceptibility to infection than those grown with an adequate nitrogen supply.

It is known that with a low level of nitrogen nutrition, carbohydrates accumulate as a result of their restricted use in the formation of organic nitrogenous compounds. On the other hand, lignin, and cellulose as well, are secretion products of protoplasm. When this secreting system is limited in extent or in activity as a result of a low plane of nitrogen assimilation, the rate of lignification may also be retarded as compared with that in tissues where the plane of nitrogen nutrition is on a somewhat higher level. This may therefore account for the relatively greater susceptibility to infection of the seedlings grown with a deficient than with an adequate nitrogen supply.

The particular level of nitrogen nutrition which would be associated with the maximum degree of resistance to infection was not determined. This level would undoubtedly vary from season to season and be dependent upon the several factors which, taken together, affect either the assimilation of nitrogen or the synthesis of carbohydrates and upon which may depend the capacity of tissues to respond to infection and wounding, by the process of cell-wall lignification.

Summary

Cucumber seedlings were grown in sand culture at high and low levels of nitrogen nutrition during several different seasons of the year. The seedlings were artificially inoculated at different ages with a fungus of the *Pythium* type, causing the damping-off disease, and the relative resistance or susceptibility to infection noted. The more important results are as follows:

1. Young seedlings were more susceptible to infection than older ones.
2. Seedlings grown under the relatively poor light conditions of winter re-

mained susceptible to infection for a longer time than those grown under the good light conditions of late spring and early summer.

3. Seedlings grown with no external supply of nitrogen remained susceptible to infection for a longer time than seedlings supplied with a complete nutrient solution containing nitrogen.

4. Resistance to infection was accompanied by a deposition of lignin in the cell walls of the tissue surrounding the area of infection. Susceptibility to infection was accompanied by the incompleteness or absence of such cell-wall lignification. It is suggested that such lignification, when continuous, may serve as a barrier in preventing spread of the fungus.

5. Deposition of lignin was found to be a function of living parenchymatous cells and occurred in the vicinity of a wound or fungus infection. In a healthy plant these cells did not become lignified.

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LITERATURE CITED

1. ATKINSON, G. F., Damping-off. Cornell Agr. Exp. Sta. Bull. 94. 1895.
2. ANDERSON, P. J., *Pythium* damping-off and root-rot in the seed bed. Connecticut Agr. Exp. Sta. Bull. 359. 1934.
3. BRAUN, H., Comparative studies of *Pythium debaryanum* and two related species from geranium. Jour. Agr. Res. 30:1043-1062. 1925.
4. BEATTIE, J. H., The production of cucumbers in greenhouses. U.S. Dept. Agr. Farmers Bull. 1320. 1923.
5. CONANT, G. H., Histological studies of resistance in tobacco to *Thielavia basicola*. Amer. Jour. Bot. 14:457-780. 1927.
6. COOK, W. S., Relation of nutrition of tomato to disposition to infectivity and virulence of *Fusarium lycopersici*. BOT. GAZ. 98:647-669. 1937.
7. DRECHSLER, C., The cottony leak of cucumbers caused by *Pythium aphanidermatum*. Jour. Agr. Res. 30:1035-1042. 1925.
8. DUGGAR, B. M., Fungous diseases of plants. New York. 1909.
9. HARTLEY, C. H., Damping-off in forest nurseries. U.S. Dept. Agr. Bull. 934. 1921.
10. HEALD, F. D., Manual of plant diseases. New York. 1933.
11. JOHANSEN, D. A., Tertiary butyl alcohol methods. Part II. El Palo Alto News 1:5-6. 1937.
12. JONES, L. R., Disease resistance of potatoes. U.S. Dept. Agr. Bur. Pl. Ind. Bull. 87. 1905.
13. JONES, L. H., and SHIVE, J. W., Influence of ammonium sulphate on plant growth in nutrient solutions and its effect on hydrogen-ion concentration and iron availability. Ann. Bot. 37:355-377. 1923.
14. MEHRlich, F. P., Medium for the growth of Pythiaceus fungi. Phytopath. 24:1127-1128. 1934.
15. McCLUNG, C. E., Microscopical technique. New York. 1929.
16. NIGHTINGALE, G. T., The nitrogen nutrition of green plants. Bot. Rev. 3:85-174. 1937.

17. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.
18. RATHBUN, A. E., Methods of direct inoculation with damping-off fungi. *Phytopath.* 11: 80-84. 1921.
19. RAWLINS, T. E., *Phytopathological and botanical research methods*. New York. 1933.
20. SHIVE, J. W., and STAHL, A. L., Constant rates of continuous solution renewal for plants in water cultures. *BOT. GAZ.* 84:317-323. 1927.
21. SMITH, W. W., The course of stone cell formation in pear fruits. *Plant Physiol.* 10:587-611. 1935.
22. SPARROW, F. K., JR., Observations on the parasitic ability of certain species of *Pythium*. *Phytopath.* 22:385-390. 1932.
23. TISDALE, W. H., Flaxwilt: A study of the nature and inheritance of wilt resistance. *Jour. Agr. Res.* 11:573-606. 1917.
24. ULLSTRUP, A. J., Histological studies on wilt of China aster. *Phytopath.* 27:737-748. 1937.
25. WATSON, B. M., BAILEY, L. H., and SEYMOUR, A. B., Damping-off. *Amer. Garden* 11: 348-350. 1890.

PHOTOPERIODIC AFTER-EFFECTS IN SIX COMPOSITES¹

VICTOR A. GREULACH

(WITH FIVE FIGURES)

Introduction

Photoperiodic after-effects were discovered in *Cosmos bipinnatus* by GARNER and ALLARD (3) and have since been observed in several other species of composites. BIDDULPH (1) found that when plants of the Klondike variety of *C. sulphureus* were transferred from short to long photoperiods, anomalies developed, including change from opposite to spiral phyllotaxy, elongation and foliation of bracts, abortive buds, twin flower heads, and elongated internodes in the region of the involucre bracts. GARNER and ALLARD did not describe such structures for *C. bipinnatus*. MURNEEK (7, 8) found that when *Rudbeckia* plants were transferred from long to 7-hour photoperiods some plants failed to bloom; some formed the usual type of flower heads (except that these were either sessile or borne on short peduncles, the plants remaining in the rosette condition); and some bore "vegetative flowers" with green petals and more or less vegetative stamens and pistils.

In an effort to determine whether similar photoperiodic after-effects occur in certain other species of composites, experiments were conducted on *Cosmos bipinnatus* Cav., *C. sulphureus* Cav. (Orange Flare variety), *Rudbeckia hirta* L., *Matricaria parthenoides* Desf., *Centaurea cyanus* L., and *Coreopsis tinctoria* Nutt. Both species of *Cosmos* were found by GARNER and ALLARD (3) to be short-day plants. However, the Orange Flare variety of *C. sulphureus*, unlike the Klondike variety, is not strictly a short-day plant. ROBERTS and STRUCKMEYER (12) reported that it acted as a short-day plant in cool temperatures and as an intermediate or neutral plant at higher temperatures, blooming under both long and short photoperiods. The species of *Rudbeckia* have generally been classed as long-day plants, but ROBERTS and STRUCKMEYER (11) found that under low night temperatures *R. laciniata* formed flower buds after a relatively long time. The other three species were reported by POESCH and LAURIE (9) to be long-day plants.

METHODS.—All plants were maintained in soil in porous clay pots. Four or five replications were made of each treatment, a total of 786 plants being used. All seeds germinated, and the seedlings began growth under photoperiods unfavorable for the initiation of flower primordia, that is, under long photoperiods for the two species of *Cosmos* and under short photoperiods for the other four species. Induction treatments were begun 28 days after planting, short induction photoperiods

¹ Papers from the Department of Botany, Ohio State University, no. 436.

being employed for the two species of *Cosmos* and long ones for the other species. After exposure to one induction photoperiod, four or five plants of each species were returned, for the duration of the experiments, to photoperiods not favoring the initiation of floral primordia. A second set of plants of each species was exposed to two, a third set to three, induction photoperiods, and so on, the maximum number being 20 for *Cosmos bipinnatus*, *C. sulphureus*, and *Centaurea cyanus*, and 30 for the other three species. One set of plants of each species was retained under photoperiods unfavorable for the initiation of floral primordia and another set under photoperiods of the length used in the induction treatments, throughout the experiments.

The experiments were conducted at Columbus, Ohio. They were begun May 18 and concluded September 3, 1939, unless otherwise noted. The plants were started in the greenhouse but were kept outside during the major part of the time. The long photoperiods, 15 or more hours, were secured by employing the natural photoperiod, either alone or supplemented with Mazda lamps. The short photoperiods, 9 hours in length, were secured by means of ventilated light-proof cabinets. The temperature differences between the inside and the outside of the cabinets were too small to have any significant influence on the development of the plants. The temperature during the experiments ranged between 60° and 90° F., except for a few brief periods, the mean temperature being 75° F.

Results

COSMOS BIPINNATUS.—By July 1, 45 days after planting, flower buds were evident on plants exposed to 9 and to 12 or more short photoperiods. Anthesis first occurred on July 9 on one plant exposed to 13 short photoperiods and on one exposed to 14. From then until August 14, one or more plants bloomed daily, with interruptions of only a day or two. Blooming was confined mostly to plants exposed to 12 or more short photoperiods, but single flower heads developed on one plant each in the groups exposed to 5, 6, and 10 short photoperiods, two heads on a plant exposed to 9 short photoperiods, and three on a plant exposed to 8 short photoperiods. Flower buds developed on a number of other plants but failed to open by the termination of the experiment, August 26. Fruits developed on plants in all groups exposed to 12 or more short photoperiods.

At the time of the last transfer from short to long photoperiods there was marked though somewhat uneven gradation in the height of the plants, those exposed to greater numbers of short photoperiods being smaller. Once under long photoperiods, all plants began growing rapidly; but the plants exposed to greater numbers of short photoperiods were smaller at the end of the experiment, not only because of their delayed start but also because their growth was checked by the formation of terminal flower buds.

Reproductive-vegetative interphases, often more extreme than those observed in *C. sulphureus* by BIDDULPH (1), occurred in abundance. The first anomaly to appear was lengthening of the lower cycle of involuclral bracts. These are usually 1 cm. or less in length in this species, but bracts as long as 9 cm. were formed. The longer bracts were often highly lobed and even foliaceous, but simple bracts 5-6 cm. long were not uncommon. Although there was little relation between maximum bract length and number of short photoperiods to which the plants were exposed, both the average and minimum bract lengths were less in the plants exposed to greater numbers of short photoperiods.



FIG. 1.—Representative floral modifications in *Cosmos bipinnatus*, August 1. Numbers refer to number of short photoperiods to which plants had been exposed. From left to right the specimens represent: flower head with elongated bracts (one separated from the others by stem elongation); two with elongated bracts in a whorl some distance below the head, bracts of first one being incised; bracts elongated but not separated from head; bracts elongated and scattered, with reversion to vegetative growth; bracts only slightly elongated and in usual position.

In about 60 per cent of the plants, internodal elongation occurred between the flower head and the lower cycle of involuclral bracts. This occurred later than bract elongation and only below heads with elongated bracts. The length of this internode varied from less than 1 cm. to 27 cm. At first such elongation occurred only in plants exposed to 9 or fewer short photoperiods, but eventually was found in the plants exposed to more than 9 short photoperiods. In some plants the bracts were in a perfect whorl (figs. 1, 2); in others only one or a few bracts were separated from the rest (fig. 2); in others stem elongation occurred between all bracts, resulting in a spiral arrangement. Some of the bracts were decurrent, and later in the experiment many divided bracts were formed. The earlier ones had only a few

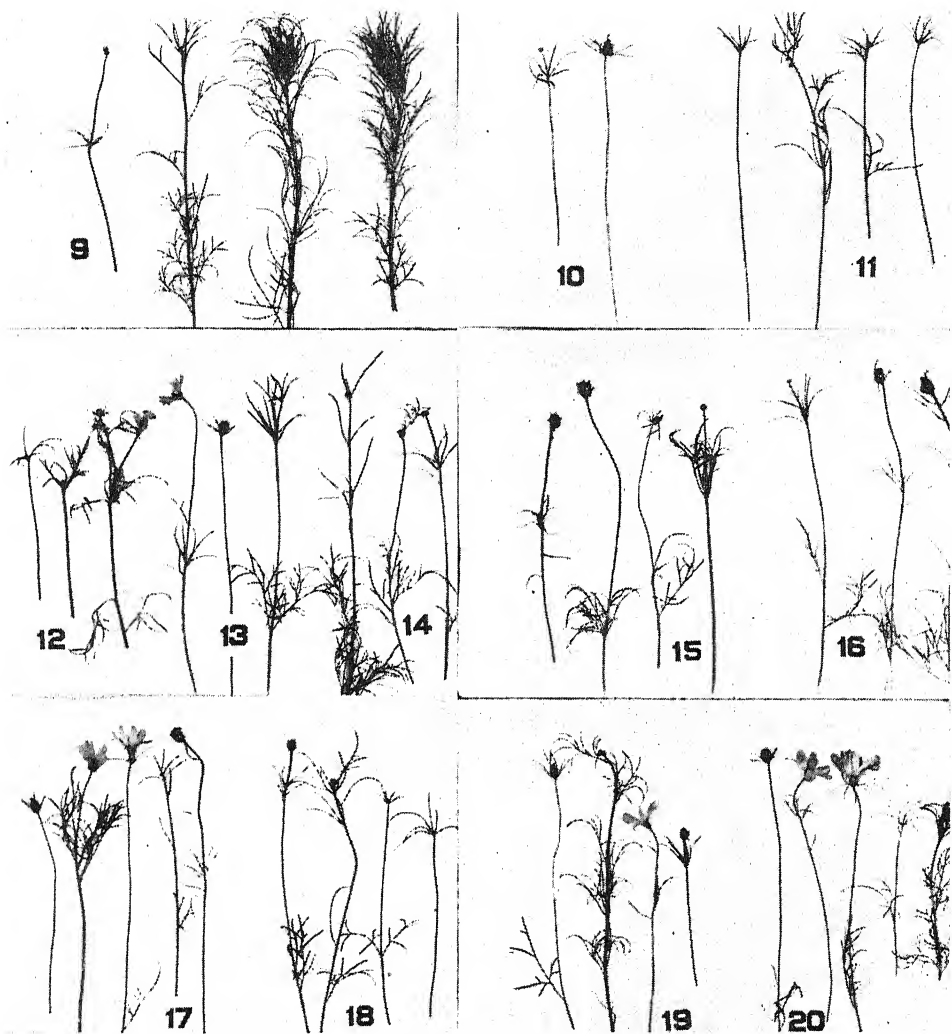


FIG. 2.—Representative floral modifications in *Cosmos bipinnatus*, August 17. Numbers indicate number of short photoperiods to which plants had been exposed. Elongated bracts shown in all the groups: some are adjacent to flower bud or head (11, two to right in 18-day group), some are arranged in whorl some distance below it (9, 10, 15, 19), and some are arranged spirally or irregularly spaced (9, 11, 14, 16). While many bracts were entire (10, 11, 18), others became incised and more or less foliaceous (9, 15, 17, 19). Some flower heads were asymmetrical and distorted (13, 17), and some flower buds were abortive (11, 14). In a few plants reversion to vegetative growth occurred (9).

lobes, but later many foliaceous bracts—resembling ordinary cosmos leaves in the complexity of their incision—were formed.

Small abortive flower buds, some barely visible, developed as the plants grew older. These were almost always subtended by long simple bracts not separated from the stem by internodal elongation (fig. 2). There were also some very small flower heads, or heads containing only a few ray flowers, some of which were foliaceous. There were no twin flower heads such as reported by BIDDULPH for *C. sulphureus*.

Actual resumption of vegetative growth occurred in several plants from terminal flower buds after the lower cycle of bracts had been formed, these usually



FIG. 3.—Portion of *Cosmos bipinnatus* plant, August 17, which had been exposed to 5 short photoperiods and on which a flower bud began forming at point where lowest bracts are located. Arrow points to single pink petaloid structure which formed before reversion to vegetative development. Later a reversion to reproductive development occurred, two flower buds lacking the lower set of bracts forming. One of these bloomed later.

being elongated and compound. The new stems had unusually short internodes, and the leaves were mostly alternate rather than opposite—as is usual in cosmos. On one plant exposed to 5 short photoperiods a flower bud formed about the middle of July but soon reverted to vegetative growth. One of the bracts of the lower cycle which had been formed was unusual in that it had one lobe which was pink, like the petals of this variety (fig. 3). The new stems not only had short internodes but were also highly branched, and the leaves were unusually small and finely divided. About August 7 two small flower buds, both lacking the usual lower cycle of bracts, formed on these stems. Their development was unusually slow, but on August 26 one finally opened into a small but symmetrical flower head. The other bud had not opened by the end of the experiment. Flower buds did not form on any of the rejuvenated branches of the other plants.

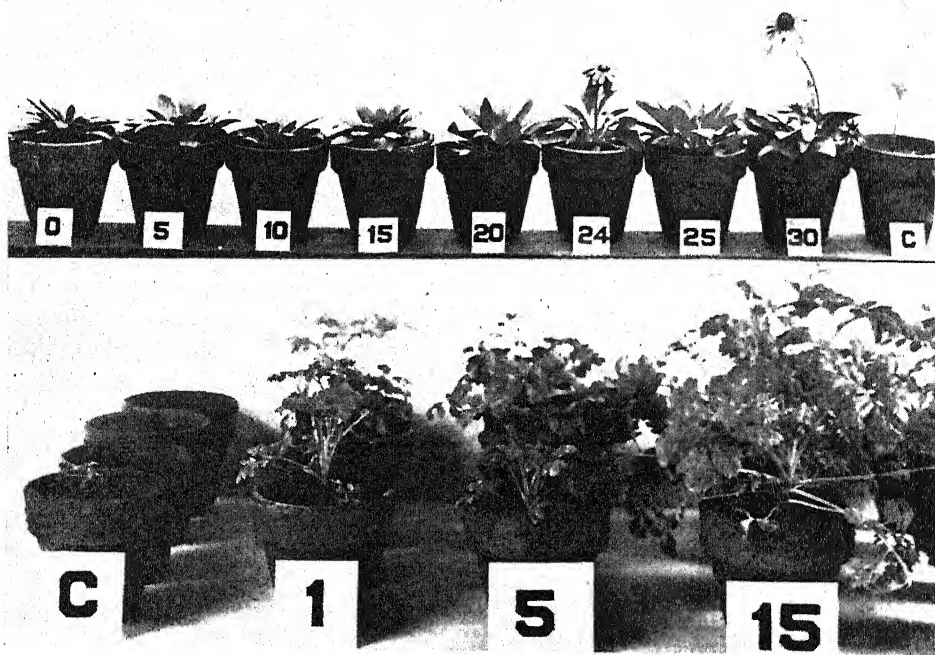
The general order of appearance of the various anomalies was as follows: bract elongation, internodal elongation, incision of bracts, defective flower heads, abor-

tive buds, and reversion to vegetative growth. These usually appeared earliest in the plants exposed to the fewer numbers of short photoperiods and appeared in increasing abundance and extremity of modification with time.

COSMOS SULPHUREUS.—This experiment extended from June 17 to August 24. Blooms first appeared on August 10, on plants exposed to 8, 9, 11, and 12 short photoperiods; and by August 14 even plants exposed to as few as 3 short photoperiods were blooming. Plants exposed to 3 or more short photoperiods continued to bloom in considerable abundance, but not so extensively as those retained under short photoperiods, which began blooming August 1 and bloomed profusely until the end of the experiment. Plants exposed to 2 short photoperiods first bloomed August 21, and on August 16 a single bloom appeared on a plant exposed to one short day. On August 20 and again on August 23 a plant retained under long photoperiods bloomed. None of the plants had anomalous bracts or floral modifications. The effect of the short photoperiods on the height of the plants was similar to that in *C. bipinnatus*.

RUDBECKIA HIRTA.—On July 20, 63 days after planting, all plants exposed to 29 or fewer long photoperiods were still rosettes, although those exposed to 9 or more long photoperiods had somewhat larger leaves than the others. The plants exposed to 30 long photoperiods were beginning to form peduncles, and those retained under long photoperiods were developing elongated stems. Flower buds were forming on plants of both these groups. On August 9 one plant exposed to 30 long photoperiods and one retained continuously under long photoperiods bloomed. By August 17 all plants retained under long photoperiods and two exposed to 30 long photoperiods were blooming, and a small flower head had formed on one plant exposed to 24 long photoperiods. By August 26 all plants exposed to 30 long photoperiods, two exposed to 27, and one each exposed to 23, 24, 26, 28, and 29, were blooming. A "vegetative flower" had developed on a plant exposed to 17 long photoperiods, a number of these also being present on other plants which had the usual type of flower heads as well. By the end of the experiment on November 4, plants exposed to 17, 23, 24, and 26 or more long photoperiods had bloomed, although the plant exposed to 17 long photoperiods bore only the single vegetative flower previously mentioned.

Only the plants retained continuously under long photoperiods developed the usual tall stems present in blooming plants of this species; they averaged 43.5 cm. in height. The other plants remained in the rosette condition (fig. 4). Those which bloomed usually had short peduncles, but one plant each in the groups exposed to 23 and 29 long photoperiods had sessile flower heads. The peduncles ranged in height from 2.0 to 16.0 cm., averaging 6.7 cm. Although the 16-cm. peduncle was developed by a plant exposed to 30 long photoperiods, there was no definite correlation between the number of long photoperiods and the lengths of the peduncles.



FIGS. 4, 5.—Figures indicate number of long photoperiods to which plants were exposed. Fig. 4 (above), representative plants of *Rudbeckia hirta*, September 1. C, plant retained under long photoperiods after 28 days from planting. Leaves of plants exposed to long photoperiods are elevated more than those of plant retained under short photoperiods. Two small “vegetative flowers” visible (below the large flower head, formed earlier) on plant exposed to 30 long days. Fig. 5 (below), representative groups of *Matricaria parthenoides* plants, August 1, showing greater size of those exposed to greater numbers of long photoperiods, and dead control plants retained continuously under short photoperiods.

The plants which failed to bloom also lacked macroscopic flower buds, but some had elevated rosette leaves, described as evidence of photoperiodic induction by MURNEEK (8).

MATRICARIA PARTHENOIDES.—Short photoperiods had a definite inhibiting effect on the vegetative development of plants of this species. By July 20 the set of plants retained continuously under short photoperiods had grown very little. There was a general increase in size of the plants exposed to long photoperiods with an increase in the number of these induction photoperiods. These inequalities gradually became less apparent, and eventually some of the plants exposed to intermediate numbers of long photoperiods developed leaves as large as those on plants exposed to the greater numbers. The plants retained continuously under short photoperiods failed to grow beyond a certain stage, however, and between July 20 and 30 all four plants of this group died (fig. 5). Their maximum height was 1.5 cm., measuring from the soil line to the tips of the leaves, while at the same time plants exposed to long photoperiods ranged from 5 to 20 cm. in height. Even the plants exposed to only one long photoperiod thrived.

By September 1 all the plants retained continuously under long photoperiods were blooming, and all had elongated stems averaging 31 cm. in height. All the other plants were still rosettes, and there was no evidence of flower buds on any of them. During September, plants in the groups exposed to 10 and 15 long photoperiods bloomed, and one plant exposed to 13 long photoperiods finally bloomed on November 2. None of the other plants bloomed, and none had flower buds or elongated stems. Blooming never occurred in this species without previous stem elongation, and none of the flowers was unusual in structure.

CENTAUREA CYANUS.—Between July 20 and 24 the plants retained continuously under long photoperiods and one plant exposed to 20 long photoperiods were exhibiting stem elongation, and by July 31 flower buds were present on all of them. On August 10 a flower bud appeared on a plant exposed to 17 long photoperiods, but this never opened. The flower head on the plant exposed to 20 long photoperiods was small and unsymmetrical. The plants retained under long photoperiods began blooming August 15 and continued to bloom in the usual manner. At this time one plant each in the groups exposed to 5, 7, and 10 long photoperiods, and most of the plants exposed to 12 or more, had elongated stems 6–16 cm. in height. These stems were unusually thick and fleshy. The plants retained continuously under long photoperiods had stems averaging 36.5 cm. in height. By the close of the experiment on October 3, plants exposed to 10, 12, 13, and 15 long photoperiods had bloomed. All plants which bloomed had elongated stems.

COREOPSIS TINCTORIA.—During the last week in July stem elongation began in the plants retained under long photoperiods, and buds began appearing on these plants August 2. By this date stem elongation was also occurring on a single

plant of the groups exposed to 18, 19, 23, 25, 28, and 30 long photoperiods. The plants retained under long photoperiods began blooming August 17 and continued to bloom profusely until the end of the experiment on September 3. One flower bud opened on August 24 and two on August 26 on a plant exposed to 18 long photoperiods, but no further anthesis occurred on this plant. Before the termination of the experiment, plants exposed to 23, 24, and 27 or more long photoperiods had also bloomed sparsely. Stem elongation was evident in one plant exposed to 4 long photoperiods, two exposed to 9, one exposed to 18, and in one or two plants of each group exposed to 20 or more long photoperiods. All plants retained under long photoperiods had elongated stems averaging 85 cm. in height, in comparison with 40 cm. for plants returned to short photoperiods. Only plants with elongated stems bloomed, and no unusual floral structures developed.

Discussion

After a plant has initiated flower primordia or a particular type of vegetative development under the influence of certain photoperiods (photoperiodic induction) and then is transferred to photoperiods not favorable for the initiation of such development, one of two things—or intergradations between them—may occur. The development may continue as a photoperiodic after-effect, or it may be inhibited (photoperiodic inhibition). When flower primordia are thus inhibited the plant may become rejuvenated; that is, vegetative development may be resumed. These phenomena should not be confused with what may be termed “obligatory photoperiodic change,” that is, the fact that in some species the photoperiods favoring the initiation of flower primordia are different in length from those necessary for the continuation of reproductive development (5), or with “photoperiodic vernalization” (10), in which photoperiods of a certain length favor the initiation and completion of reproduction but must be preceded by photoperiods of a different length or some other vernalizing agent which favors production of “ripeness to flower.”

In the present study photoperiodic after-effects were evident in all six species, as indicated by the formation of flower buds, flower heads, and in some species fruits, following transfer of the plants to photoperiods not favoring the initiation of flower primordia from photoperiods favoring such initiation. These after-effects were much less extensive and occurred only after exposure to a greater number of induction photoperiods in the long-day than in the short-day species. This may eventually be found to be a more or less general difference between long- and short-day species. Although HAMNER and NAYLOR (4) observed after-effects in dill plants exposed to only a few long photoperiods, MURNEEK (8) later pointed out that dill will eventually reproduce under short photoperiods and should conse-

quently be considered a neutral or intermediate rather than a true long-day species.

Short photoperiods retarded the vegetative growth of both the long- and short-day species studied. This was most extreme in *Matricaria*, marked stunting and subsequent death occurring in the plants retained continuously under short photoperiods, while plants exposed to even one long photoperiod thrived, although they did not become so large as those exposed to more long photoperiods. There was no clear case of the persistence of short-day inhibition of vegetative growth as an after-effect. Although the independence of photoperiodic after-effects as regards flower formation and photoperiodic inhibition of stem elongation reported by MURNEEK (7, 8) for *Rudbeckia* was evident in *R. hirta* in this study, it did not occur in any of the other long-day species and obviously could not occur in the short-day *Cosmos* plants. In these other long-day species stem elongation occurred in some of the plants exposed to greater numbers of long induction photoperiods and may be considered a photoperiodic after-effect. Although these stems were not so tall as those of plants retained continuously under long photoperiods, only plants with elongated stems bloomed. Apparently stem elongation and flower-head formation are not independent in all long-day composites.

The vegetative flowers of *Rudbeckia* and the anomalous bracts and flower heads of *Cosmos bipinnatus* may be considered as evidence of partial reversion to vegetative development (rejuvenation), while in the latter species actual resumption of vegetative growth from flower buds occurred in some plants. This is in the same category as the rejuvenation of hemp observed by SCHAFFNER (13). The anomalous structures in *C. bipinnatus* represent a graded series of reproductive-vegetative interphases: elongation of bracts, stem elongation between bracts, incision and foliation of bracts, formation of defective flower heads and abortive buds, and finally actual resumption of vegetative growth from the flower bud. Except for the vegetative flowers of *Rudbeckia*, no such interphases occurred in the other species. Their absence in *Cosmos sulphureus* was evidently due to the fact that long photoperiods in conjunction with relatively high temperatures are favorable for reproductive development in the Orange Flare variety used. In view of the scanty after-effects in *Matricaria*, *Coreopsis*, and *Centaurea*, numerous interphases might be expected, but none occurred. This seems to indicate that such interphases are not produced by all species of composites.

Since the anomalous bracts and flower heads in *Cosmos bipinnatus* occurred earliest in the plants exposed to the smallest numbers of short photoperiods and became more pronounced with time, they tend to add weight to CHAILAKHIAN'S (2) theory of florigen charge—that during induction a charge of florigen accumulates and is gradually used after transfer to photoperiods not favorable for initia-

tion of primordia. They would be more difficult to explain if we accept the theory (6) that during induction there is a permanent change in metabolic processes which makes possible the continuation of florigen synthesis, even after transfer.

Since the short induction photoperiods greatly increased the abundance and earliness of bloom in the Orange Flare *Cosmos*, apparently it should not be considered a strictly neutral or intermediate plant at high temperatures, even though it can initiate flower primordia under both long and short photoperiods at such temperatures. In this variety short photoperiods are more effective than long ones in initiating flower primordia, even at higher temperatures.

Summary

1. Photoperiodic after-effects were observed in two short-day species (*Cosmos bipinnatus*, *C. sulphureus*) and four long-day species (*Rudbeckia hirta*, *Matricaria parthenoides*, *Centaurea cyanus*, *Coreopsis tinctoria*). Twenty-eight days after planting the seed, the plants being kept under photoperiods unfavorable for reproductive development, they were subjected to from 1 to 20 or 30 induction photoperiods of a length which favored initiation of flower primordia. They were then transferred to photoperiods not favoring such development and retained there for the duration of the experiments.

2. In *Cosmos bipinnatus* anthesis occurred in some plants exposed to 5-11 short photoperiods, and in abundance in all plants exposed to 12 or more short photoperiods. In the Orange Flare variety of *C. sulphureus* even the plants retained continuously under long photoperiods eventually bloomed sparsely, but the abundance of flowers increased with an increase in the number of short induction photoperiods. Plants of the long-day species *Rudbeckia hirta*, *Matricaria parthenoides*, *Centaurea cyanus*, and *Coreopsis tinctoria* bloomed sparsely after exposure to minima of 17, 10, 10, and 18 long photoperiods, respectively.

3. Photoperiodic inhibition of stem elongation by the short photoperiods of the transfer environment occurred in *R. hirta*, even in the plants which bloomed. In the other three long-day species such inhibition of stem elongation was not universal, and only plants with elongated stems bloomed.

4. The short photoperiods inhibited the vegetative growth of both the long- and short-day species. In *Matricaria* the plants retained continuously under short photoperiods reached a total height of only 1.5 cm. and soon died, while plants exposed to as little as one long photoperiod thrived.

5. Reproductive-vegetative interphases, which may be considered as initial stages in rejuvenation, occurred in two species. A number of "vegetative flowers" with green petals and more or less vegetative stamens and pistils developed on plants of *Rudbeckia hirta*. In *Cosmos bipinnatus* there were many elongated bracts, incised and foliaceous bracts, elongated internodes between bracts, and defective

flower heads and abortive buds. Some plants of this species actually resumed vegetative growth from young flower buds. One plant exposed to 5 short photoperiods made a double reversal in its terminal bud, changing from vegetative to reproductive growth, back to vegetative growth, and once more to reproductive growth.

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LITERATURE CITED

1. BIDDULPH, O., Histological variations in cosmos in relation to photoperiodism. *BOT. GAZ.* 97:139-155. 1935.
2. CHAILAKHIAN, M. K., Concerning the hormonal nature of plant development processes. *Compt. Rend. Acad. Sci. U.R.S.S.* 16:227-230. 1937.
3. GARNER, W. W., and ALLARD, H. A., Further studies in photoperiodism: the response of the plant to the relative length of day and night. *Jour. Agr. Res.* 23:871-920. 1923.
4. HAMNER, K. C., and NAYLOR, A. W., Photoperiodic responses of dill, a very sensitive long-day plant. *BOT. GAZ.* 100:853-861. 1939.
5. LOEWING, W. F., Photoperiodic aspects of phasic development. *Science* 90:552-555. 1939.
6. LUBIMENKO, V. N., and SZEGLOVA, O. A., Sur l'induction photopériodique dans le processus du développement des plantes. *Bull. Bot. Gard. Leningrad* 1-2:1-52. 1931.
7. MURNEEK, A. E., A separation of certain types of response of plants to the photoperiod. *Proc. Amer. Soc. Hort. Sci.* 34:507-509. 1936 (1937).
8. ———, Length of day and temperature effects in *Rudbeckia*. *BOT. GAZ.* 102:269-279. 1940.
9. POESCH, G. H., and LAURIE, A., The use of artificial daylight and reduction of the daylight period for flowering plants in the greenhouse. *Ohio Agr. Exp. Sta. Bull.* 559. 1935.
10. PURVIS, O. N., and GREGORY, F. G., Studies in the vernalisation of cereals. A comparative study of vernalisation of winter rye by low temperature and short days. *Ann. Bot. N.S.* 1:569-591. 1937.
11. ROBERTS, R. H., and STRUCKMEYER, B. E., The effects of temperature and other environmental factors upon the photoperiodic responses of higher plants. *Jour. Agr. Res.* 56:633-678. 1938.
12. ———, Photoperiod, temperature and some hereditary responses of plants. *Jour. Hered.* 29:95-98. 1938.
13. SCHAFFNER, J. H., The change of opposite to alternate phyllotaxy and repeated rejuvenation in hemp by means of changed photoperiodicity. *Ecology* 7:315-325. 1926.

INTERRELATION OF ORGANIC MATERIALS IN THE GROWTH SUBSTANCE RESPONSE

S. C. BAUSOR

(WITH FOUR FIGURES)

Introduction

The manner in which growth substances influence cellular activity is one of the more pressing problems in plant physiology. The diverse effects induced by these substances and the low concentrations in which they are effective suggest that their function may be regulatory, but not in a specific way. At least no single effect can be ascribed to them. The response is of a general nature, and normal cellular activities are accelerated or depressed. Mature cells may be rejuvenated to renewed activity, their relation to neighboring cells and tissues may be altered, and they may assume embryonic qualities such as enlargement, increment in protoplasmic content (especially marked in the nucleus), and mitoses. This chain of events is often followed by organization of new structures such as roots or shoots, or merely galls, some of the cells of which may mature as vascular strands. Occasionally mature cells do differentiate, or differentiate differently from normal, as in certain parenchymatous cells of the pith described later which become reticulately thick walled. Apparently the key to the problem of senescence is to be found in the reaction of the growth substances.

Literature review

The object of the present work was to determine the histological and cytological changes resulting from the interaction of growth-regulating substances and nutrients supplied to cuttings. Many researches have already appeared which shed some light upon the role played by growth-regulating substances in the metabolism of the plant.

There is considerable increase in water content in tissues affected by growth substances. STUART (29) reported an increase in fresh weight of bean cuttings accompanied by an increase in pectic compounds, when treated with indoleacetic acid in lanolin, and MITCHELL (16) found an increased percentage of water in regions of bean plants treated with lanolin mixtures of naphthaleneacetic acid or the amide.

In respect to dry matter, MITCHELL and HAMNER (17) found an increase in dry weight of beans treated with application of high dilutions of indoleacetic acid in lanolin. On the other hand, with high concentrations of growth substance less or-

ganic matter was synthesized than in the controls, according to the researches of ALEXANDER (1) using indoleacetic acid and MITCHELL (16) comparing naphthaleneacetic acid and naphthalene acetamide. MITCHELL, however, discovered that the nitrogen content was appreciably higher in the treated plants than in the controls, but the distribution of the nitrogen was different with respect to the two compounds. The gall formed at the decapitated apex in plants treated with naphthaleneacetic acid contained about a third of the total nitrogen, but in the case of the acetamide, where lignification is a characteristic response (13), only slightly more nitrogen accumulated in the apical region than in the controls.

Another effect of the growth substances is the mobilization of materials toward the regions of vigorous growth and a redistribution of storage materials in the plant. The accumulation of nitrogen in the apical gall just mentioned is a case in point. ALEXANDER (1) found a similar movement of carbohydrates toward the apical swelling and an accumulation of starch below it. MITCHELL and MARTIN (19) indicated that the direction of flow of materials from the cotyledons of etiolated bean plants was determined by indoleacetic acid.

STUART and MARTH (30) observed an accumulation of sugars in the stems of *Ilex opaca* cuttings when treated with indolebutyric acid. BORTHWICK, HAMNER, and PARKER (5) showed by microchemical tests an increase in proteins and a decrease in starch in regions of the tomato stem where cell divisions were initiated by indoleacetic acid. Starch accumulated below this region.

STUART (29) showed the mobilizing effect of indoleacetic acid upon bean cuttings up to the time of root emergence, in which the rate of movement of materials from the leaves to the hypocotyl was greatly accelerated. Dry weight, nitrogen content, and pectin increased in the hypocotyls over the controls. Sugars at first accumulated faster in the hypocotyls of the treated plants but later became less than in the controls. After root emergence, MITCHELL and STUART (20) found that nitrogen decreased in hypocotyls of experimental and control plants, but much more rapidly in the former. They concluded that indoleacetic acid affected the proteolytic enzymatic action.

Mobilization seemed to be effected through the action of the growth substances on the enzymatic system. MITCHELL, KRAUS, and WHITEHEAD (18) showed that naphthaleneacetic acid sprayed upon the leaves of kidney bean plants hastened the hydrolysis of starch and dextrans in the leaf. Sugars accumulated at first but later fell to the level of the controls. Leaves depleted of starch and dextrans by being kept in the dark accumulated less starch, dextrans, and sugars when placed in the light and sprayed than did the controls. MITCHELL and WHITEHEAD (21) extended this investigation, reporting that hydrolysis of starch followed upon spraying bean leaves with indoleacetic, indolepropionic, indolebutyric, naphthaleneacetic, and naphthoxyacetic acids. A lesser effect on starch was obtained with phenyl-

acetic acid, and none at all with naphthalene acetamide. The increase of sugars in holly stems (30) presumably resulted from the hydrolysis of starch in the leaves, since only a trace of starch was present in the stems. BEAL (3) illustrated the disappearance of starch from the endodermis of bean stems treated with indoleacetic acid.

The interrelation of other materials with the growth substances has been reported in several publications. FLIRY (10) demonstrated the resumption of elongation in decapitated sunflower hypocotyls by the application of either indoleacetic acid or sugars (sucrose and glucose) to the cut surface. SWEENEY and THIMANN (31) showed that the transient effect of streaming in the protoplasm of *Avena* epidermis could be continued for 2 hours in a 1 per cent solution of fructose. SCHNEIDER (26) reported increased growth of sections of oat coleoptiles when sucrose, maltose, dextrose, levulose, or endosperm extract was added to the test solution of indoleacetic acid. Mannitol appeared to be without effect. Both sugar and indoleacetic acid in the medium gave better results than either alone.

COOPER (6, 7, 8), working on root responses, especially of *Citrus* cuttings, observed that if the end of the cuttings, treated with an aqueous indoleacetic-acid solution, were removed the effect of the growth substance was voided and further application of the acid did not lead to the formation of more roots than the controls. He concluded that another substance, rhizocaline, besides the growth substance was necessary for root formation. Failure to bring about rooting in apple cuttings was explained as due to deficiency in rhizocaline. WENT (32), in an elaborate series of experiments with etiolated pea seedlings, proposed the interaction of different specific growth substances, the calines, with auxin in such different growth responses as stem and petiole elongation, root formation, and development of the leaf. BONNER, HAAGEN-SMIT, and WENT (4) showed that an aqueous extract obtained by leaching the cotyledons of peas and other plants greatly increased growth of the mesophyll of the leaf. The solids of the diffusate contained more than 50 per cent carbohydrates, but the accelerated growth of the leaf tissues was not a sugar effect, since the tissues were cultured in 1 per cent sucrose. WENT later (33) suggested that light was necessary for the activity of phyllocaline.

A relation has been shown to exist between the growth substances and some of the micro-elements. EATON (9) reported that boron could be replaced by indoleacetic acid in the nutrition of cotton plants and suggested the formation of auxin as one of the functions of boron. SKOOG (27) recorded an unpublished work of HOAGLAND in which indoleacetic acid seemed to improve the stunted growth of zinc-deficient plants. SKOOG found the absence of auxin in terminal buds and stem sections of zinc-deficient plants and a lower auxin content in their leaves. A decrease in auxin content occurred before the symptoms of zinc deficiency were apparent, while an increase in auxin was detected after zinc salts were added to the

nutrient of the deficient plants. He determined that indoleacetic acid sprayed on the leaves increased stem growth only in the early stages of deficiency and concluded that zinc was necessary in the metabolism of the plant to keep the growth substances in an active state and to prevent their oxidation.

Material and methods

Plants of Bonnie Best tomato and Calapproved kidney bean were used as material. In the experiments employing potted tomato plants, these were about 5 inches high, with three expanded pinnate leaves. Those from which the cuttings were taken were larger, but all cuttings were made 5 inches long and had the three uppermost leaves expanded. Bean cuttings were taken from young plants just above the second node (paired unifoliate leaves). In these the second trifoliate leaf was just expanding.

The mineral solutions used for root cultures by ROBBINS and SCHMIDT (25) and by WHITE (34) were both found in preliminary tests to be satisfactory for keeping the cuttings alive. A modification of WHITE's nutrient medium was used in all experiments reported here, in which potassium iodide, glycine, thiamin, and sucrose were omitted. To this solution a weighed amount of the material under examination was added. The solutions were changed every second day to prevent the growth of molds and the effect of fermentation products.

The cuttings were kept in the dark for 24 hours before being treated with the growth substances and remained in darkness for the duration of the experiment. The growth substances were applied unilaterally to the stems as a 1 per cent lanolin mixture. The temperature of the darkroom was 68°–70° F. The period of treatment with the growth substances in the presence of a special nutrient was 6 days, except when otherwise indicated. At the end of this time fixations were made in Allen and Wilson's modification of Bouin's solution. Controls consisted of hormone-treated cuttings in the mineral solution alone and untreated cuttings in the special nutrients.

Microscopic examination was made of the stem tissues to determine the changes resulting from the interaction of the growth substance and the nutrient supplied to the cuttings. When the reaction was great the typical white swollen areas could be seen macroscopically. For cytological and histological studies, hand sections were stained with iodine in 95 per cent alcohol, counterstained in gentian violet in absolute alcohol, cleared in xylol, and mounted in Canada balsam.

Experimental results

TOMATO

Both intact plants and cuttings that remained in darkness during the period of treatment failed to produce the typical proliferations and root initiation which

occur in plants kept in the light. As judged by curvatures, however, stretching growth was not appreciably affected.

ROOTED PLANTS.—Potted tomato plants were treated unilaterally with 1 per cent β -naphthoxyacetic acid and the controls with pure lanolin. To assure results as nearly comparable as possible, the paste was applied on small cardboard strips $\frac{1}{4} \times \frac{1}{8}$ inches, containing approximately equal quantities of the paste. The cards were applied to the middle of the third internode, in line with the uppermost expanded pinnate leaf (the third). With the exception of the curvatures, no apparent differences could be noted between treated and untreated stems after 6 days; but closer examination showed that enlargement of the nuclei took place where the tissues were activated by the growth substance. The large size of the nucleolus was characteristic. In untreated sectors opposite the paste and in comparable regions of untreated plants the nuclei were much smaller and had small nucleoli (figs. 1, 2). This was the sum total of the observable activity induced by the growth substance under these conditions. The same treatment in the light produced the typical white patches indicative of proliferation and root development.

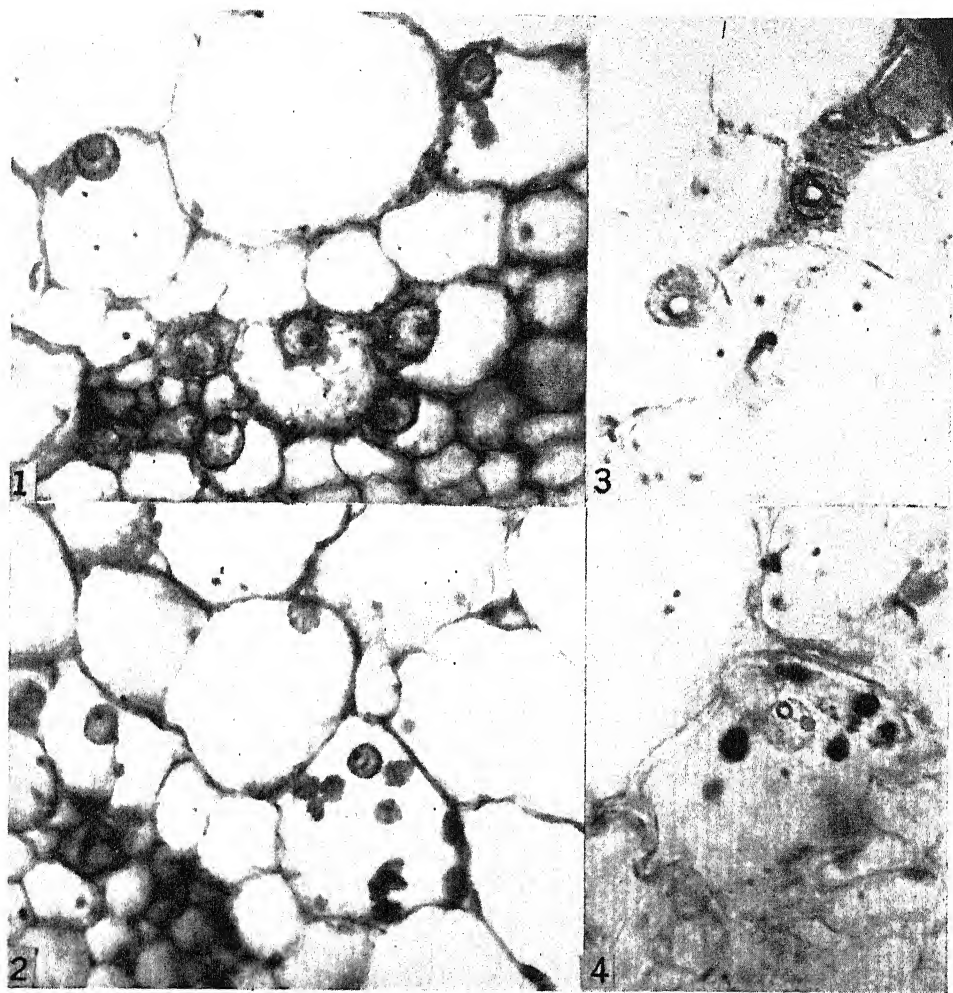
To determine what influence the foliage may have exerted in this reaction, three series of plants treated as just described were set up as follows: (a) all leaves removed except the third pinnate one, and plants decapitated; (b) all leaves removed, and plants decapitated; and (c), as in (b), but not decapitated. In series (a) the results were similar to those with intact plants, but in (b) and (c) no nuclear enlargements occurred.

The magnitude of the curvatures in the different series could not be determined accurately, since defoliation in these plants produced curvatures before the growth substance was applied.

CUTTINGS.—In all cuttings the lanolin mixture was applied copiously to one side of the stem by means of a glass rod.

Leafy cuttings maintained in the mineral nutrient showed the same cytological changes as the potted plants. Great enlargement of nuclei and nucleoli occurred (fig. 3) as compared with untreated controls. In the latter, very small nuclei were present, some with small, granule-like, refractive nucleoli, others without distinguishable ones.

In leafless cuttings the nuclei were very small in both experimental plants and controls, and the only apparent differences seemed to be the greater predominance of enucleolate nuclei in the controls. The leafy controls were similar to the treated defoliated cuttings. The growth substances had very little effect upon the metabolism of the tissues under these conditions. The results were the same with indoleacetic acid and β -naphthoxyacetic acid. The effectiveness of the growth substance apparently was dependent upon something diffusing from the leaves.



FIGS. 1-4.—Figs. 1, 2, cross-sections of stems of intact tomato plants: Fig. 1, treated with 1 per cent β -naphthoxyacetic acid in lanolin; enlargement of nuclei and nucleoli in cells of endodermis and phloem parenchyma. Fig. 2, control, treated with pure lanolin; small nuclei and nucleoli. Fig. 3, section of stem of tomato cutting in mineral nutrient, treated 6 days in darkness with 1 per cent β -naphthoxyacetic acid in lanolin; enlargement of nuclei and nucleoli. Fig. 4, tomato cutting treated in darkness 6 days with 1 per cent indoleacetic acid in lanolin; enlarged binucleolate nucleus, showing two types of nucleoli.

To investigate the limitation of the growth response in the dark, and its further limitation in defoliated plants, similar cuttings were cultured in the mineral nutrient to which 1 per cent sucrose was added and treated with 1 per cent indoleacetic acid in lanolin. The response of both leafy and defoliated cuttings followed the sequence occurring in plants in the light. Large white macroscopic areas were conspicuous. Typical proliferations occurred in endodermis, pericycle and phloem parenchyma, and cambium, and numerous root primordia developed. Enlargement and division of cells occurred in cortex and pith. Large nucleoli typical of very active cells prevailed. The activity in defoliated cuttings was somewhat less than in leafy ones, which may have been due to the decreased transpiring surface.

The controls in sucrose showed no unusual development, and no differences could be detected between those with leaves and those without. No proliferations occurred, and nucleoli were small in the cells of endodermis, pericycle, and phloem. Evidently the carbohydrate was the immediate limiting factor in the inhibition of the expected response to the growth substance. This was shown again to be the case in the following experiment.

Tomato cuttings were placed in media plus and minus sucrose, and kept in darkness for progressively longer periods before treatment with β -naphthoxyacetic acid in lanolin. Each group was subdivided into six and treated after 1, 2, 3, 4, 5, and 6 days. All series were subjected to the growth substance for 6 days and differed from one another only in the length of time in the dark before treatment.

The response was similar in all plus-sucrose cuttings, whether treated after 24 hours or after 6 days in darkness. Proliferations were abundant, and numerous root primordia developed. Nuclei, and their nucleoli especially, enlarged to a great size.

Cuttings without sucrose responded progressively less vigorously as the time before treatment was lengthened. After the 24-hour period cuttings showed enlargement of many of the nuclei, with accompanying increase in size of nucleoli in the cells of the endodermis, pericycle, phloem, and cambium. Treated after 2 days in the dark, fewer enlarged nuclei were present in pericycle, phloem, and cambium, while only an occasional endodermal nucleus was found enlarged. Those treated after 3 days in darkness had still fewer enlarged nuclei; and, as in the preceding series, these occurred mostly in pericycle and phloem. Plants treated after 4 days in the dark had no enlarged nuclei. The nucleoli, however, were definite though small and appeared as refractive granules. After 5 days the nuclei were still smaller, and with very small nucleoli which were sometimes difficult to distinguish. By the sixth day nucleoli were not visible.

The condition in cuttings treated after the fourth and fifth days in darkness was similar to that in treated and untreated defoliated cuttings, and in untreated

leafy cuttings after 1 day. Apparently the reserves or assimilated materials of the leaves—and probably also of the stem—were made available to the tissues activated by the growth substances, even under conditions of starvation. Carbohydrates were mobilized in amounts sufficient to result in nuclear enlargements and a synthesis of nucleolar substance. This occurred even after 3 days in the dark before treatment, although fewer nuclei were involved. The disappearance of the nucleolus was delayed in the activated cells until after the cuttings were in darkness for 11 or 12 days. This is almost double the time in which all nucleolar material was used up in untreated or defoliated cuttings.

When carbohydrates were available, one of the first visible effects of the growth substance was the accumulation of proteinaceous reserves in the nucleolus. These were utilized under conditions of inanition.

Enlarged nucleoli were of two types. In some the stainable substance was at the periphery, surrounding a clear central body or vacuole. Occasionally small clear areas were to be found in this peripheral material. In others the stainable material made up the whole body of the nucleolus, and scattered within were numerous (five, six, or more) small clear regions. In binucleolate nuclei both types may be represented (fig. 4).

Curvatures occurred in all the plus-sucrose plants, and in the minus-sucrose plants up to the fifth day in darkness.

An experiment was then devised to determine whether the potentialities of the nuclei were impaired by the presence of a high concentration of growth substance when a limiting supply of carbohydrate was available.

Two sets of tomato cuttings were placed in nutrient solutions, one without a carbohydrate and the other containing 1 per cent sucrose. The plants in the minus-sucrose medium were treated with 1 per cent indoleacetic acid in lanolin, after being in the dark for 24 hours, and remained in this nutrient for 3 additional days. At the end of this time they were transferred to a plus-sucrose medium, and at the same time the plus-sucrose plants, which up to now remained untreated, received an application of the indoleacetic-acid mixture. Five days later treated stems of both series were fixed for study. A concurrent set of cuttings in sucrose treated after 24 hours in darkness was also fixed at this time.

Macroscopic differences in the three sets of cuttings were conspicuous. Both sets cultured from the beginning in media containing sucrose had conspicuous white swellings, which were clearly larger in those treated with the growth substance after 1 day in the dark than in those treated after 4 days. On the other hand, the experimental lot transferred to sucrose after 4 days' treatment showed external evidence of reaction in only two of the ten cuttings. Except for these two, microscopic examination showed less activity in the treated regions than did treated cuttings maintained from the start in the medium containing sucrose. But

the response was not slight in any of the cuttings. Proliferations and root initials were abundant. Thus the typical response was shown, upon the addition of sugar to the medium, by cells containing a relatively high concentration of growth substance and low carbohydrate supply, but to a somewhat less degree than those containing a high carbohydrate supply, derived from the medium, at the time the growth substance was applied.

INTERACTION OF OTHER CARBOHYDRATES. —A comparison of the effectiveness of six sugars in relation to the response to growth substances was made by supplying them to the cuttings as in previous experiments. Three disaccharides and three monosaccharides were used in 1 per cent concentrations: sucrose, maltose, lactose, d-glucose, d-levulose, and d-galactose. All except galactose, which was toxic, were utilized by the cuttings and the typical reaction to the growth substance followed. A semi-quantitative determination of the response was made by comparing the extent of proliferation of endodermis, pericycle, phloem, and cambium; the number of root primordia induced; and the degree of activity in the pith. Three growth substances, indoleacetic acid, β -naphthoxyacetic acid, and ethyl β -naphthoxyacetate, were employed with each of the sugars.

The greatest response was obtained by the use of maltose; levulose and sucrose were only slightly less efficient. Smaller responses were obtained with glucose and lactose in approximately the same degree. Qualitatively the response was similar with all five sugars and conformed to the previous report on Marglobe tomatoes (2). In none of the controls in the different sugars was there any activity. One per cent mannitol was not utilized by the cuttings, judging from the absence of response to β -naphthoxyacetic acid.

RESPONSE IN RELATION TO NITROGENOUS CONSTITUENTS. —Tomato cuttings, cultured in a medium containing sucrose, in which nitrogen was omitted by substituting calcium sulphate for calcium nitrate and potassium nitrate, responded to treatment with the growth substances in no different manner from those in the complete nutrient. Both exhibited the full effects of the growth substance in the presence of a carbohydrate supply. Apparently there was sufficient nitrogen in the cuttings that was easily translocated to the activated tissue; and it leaves open to question the actual utilization of inorganic nitrogen in a nutrient containing it.

NITROGEN-DEFICIENT PLANTS. —Eight tomato plants grown in a minus-nitrogen mineral solution for 1 month, and eight grown in plus nitrogen, were available. The minus-nitrogen plants were very hard. They were 4 inches tall with three small yellow-green leaves. The plus-nitrogen plants were 2.5 feet high, dark green, and rather succulent. Treatments were made on the intact plants in the greenhouse, two of each series with indoleacetic acid and two with β -naphthoxyacetic acid in lanolin. Four served as controls, two lanolin treated and two untreated.

Both plus- and minus-nitrogen plants curved negatively in response to indoleacetic acid and positively to β -naphthoxyacetic acid. Portions were fixed 6 days after treatment.

The nitrogen-deficient plants responded with vigor. Proliferations occurred in the cortex, endodermis, pericycle, and phloem. Root primordia were initiated and several large roots emerged. Nuclei and nucleoli increased to a great size in all activated tissues. The high-nitrogen plants responded only slightly more vigorously. More root initials were formed and larger roots were more abundant. The controls showed no activity, nuclei were smaller than in those treated with the growth substance, and cell division was not apparent.

Eight spindly, nitrogen-deficient tomato plants, about 2 feet high and with light green foliage, were also available. They had grown in a nitrogen-free medium for 8 weeks. Cuttings 5 inches long were taken from their tops. These were placed in a nutrient medium containing 1 per cent sucrose, four plus nitrogen and four minus nitrogen; and after 24 hours in the dark were treated with indoleacetic acid in lanolin. Both reacted similarly, which again questions the utilization of the nitrates by the cuttings. Responses like those of the nitrogen-deficient plants in the light resulted from the hormone treatment.

UREA AND GLYCINE.—One per cent urea or glycine added to the nutrient solution was toxic to tomato cuttings. These were tried in order to determine whether organic nitrogenous compounds could be substituted as respiratory and structural materials in place of the carbohydrates. Treated portions of some of the plants in the nutrient containing urea were fixed 3 days after treating them with indoleacetic acid, in which time the leaves and apex had wilted but the stems to which the paste was applied appeared normal. Sections of these showed that no local activation had occurred.

KIDNEY BEAN

For comparative purposes the methods used for the tomato cuttings were repeated with the beans, with similar results. The growth response in the dark without an external source of carbohydrate was greatly increased upon the addition of sucrose to the medium. The growth substance was applied unilaterally to the lower part of the third internode.

Without added sugar, nuclear enlargement with accompanying increase in size of nucleolus took place in tissues beneath the paste. In the lower portion of the treated internode (near the node) the endodermis enlarged and divided a few times, and in some cuttings early stages in root initiation occurred at the rays. Any differences between intact and defoliated cuttings were not obvious. The lower portion of the internode gave a greater response than the rest of the internode, even in defoliated cuttings left in darkness 48 hours before treatment, which indi-

cated a higher local supply of sugars in this region of the stem. The amount of activity in intact and defoliated cuttings was undoubtedly due to their large size and to the abundance of storage starch in the stem.

When sucrose was added to the nutrient, great proliferation occurred throughout the treated area and large primordia developed. Galactose was also toxic to bean cuttings and showed the same symptoms as in the tomato.

The response to the growth substances in cuttings of etiolated bean seedlings, maintained in various organic media, was studied. The results will be deferred to a later report. Galactose and glycine, which were toxic to the green cuttings, were not injurious to the etiolated plants. The leaves and apex died in cuttings cultured in the nutrient containing 1.0 per cent urea, but 0.1 and 0.01 per cent concentrations were not toxic.

Discussion

The interrelation between the carbohydrates and the growth substances, as indicated in this work, shows how closely the two are related in the anabolic metabolism of the cell. In the presence of sugars alone mature cells remained in equilibrium and utilized the sugars apparently for respiratory purposes alone. With a quantity of growth substance present at the same time, the carbohydrates were assimilated and protein synthesis occurred. Under the conditions of the experiments, proteins apparently were resynthesized locally from materials present in other parts of the cuttings not directly affected by the growth substances. The disappearance of stainable nuclear material and the nonutilization of supplied nitrates bears this out. Conversely, considerable material accumulated in the nucleus—and especially in the nucleolus—of cells stimulated by the growth substance.

The high starch content of nitrogen-deficient plants may be due to a deficiency of natural growth substances. This is mere speculation, based upon the strong response of such plants, by both curvatures and histological changes; upon the unusual amount of growth occurring in nitrogen-deficient plants when placed in total darkness (22); and upon the effect of growth substances on the hydrolysis of starch. In nitrogen-deficient plants nitrogen does not appear to be the factor limiting growth directly, but lack of vigor may be attributable to the inability of the plant to utilize carbohydrates properly.

Nitrogen is reutilized in the plant (22, 23) and is readily available to regions activated by the growth substance. It also seems to be needed in relatively small amounts for growth in locally activated cells. For these reasons it is not likely to be a limiting factor in the rooting response of cuttings, or in other phenomena where high metabolic activity is taking place locally. Since normal development of the higher plants is a succession of local growth phenomena, the ease with which

nitrogen is reutilized must be a stabilizing factor. On the other hand, carbohydrates are apparently needed in larger quantities, and the extent of activity is determined by their availability. In this connection, SMITH, NASH, and DAVIS reported (28) that low nitrogen-high carbohydrate bean seedlings treated with indoleacetic acid rooted faster than high nitrogen-low carbohydrate plants. They concluded that "New root primordia were largely influenced by the carbohydrate reserves in the plant."

It was shown that removal of the foliage of intact tomato plants and cuttings completely inhibited the small response in these plants to the growth substances in the dark and that the presence of sugars brought about a complete response. A similar case of a weak reaction (gall production) or none at all has been reported (14) in tomato and *Bryophyllum* plants that were defoliated and decapitated, when inoculated with an attenuated strain of the crown-gall organism. Leafy plants produced larger galls in all cases. This was attributed to the diffusion of a growth substance from the leaf, in spite of the fact that 3 per cent indoleacetic acid in lanolin added to the inoculated tissues only slightly increased the amount of proliferation. It seems likely that the difference in vigor between intact and defoliated plants and the difference in the accessibility of carbohydrates are the probable explanations of these results.

The toxicity of galactose is a puzzling problem in plant nutrition. It has been investigated carefully by KNUDSON (11, 12) and others and reviewed by MILLER (15).

It seems highly probable that more vigorous root induction and better subsequent growth of young roots may be had by pretreating cuttings with a solution of an inexpensive sugar such as sucrose or glucose before using a growth substance. OLIVER (24) found that honey stimulated rooting of *Chrysanthemum* and *Thuja*. Any treatment tending to convert stored polysaccharides into sugars may have the same effect. It follows that cuttings should have the maximum number of leaves compatible with retention of water. The available carbohydrate supply may partially explain seasonal variations in the rooting of cuttings.

Further research may show the way to increased crop yields through more efficient utilization of nitrogenous compounds in particular, and also of the carbohydrates, within the plant.

Summary

1. Intact tomato seedlings and cuttings in a mineral nutrient, kept in darkness during treatment, produced only a small response to the growth substances in 6 days. This was limited to slight enlargement of the cells and an increase in size of nuclei and nucleoli.

2. The reaction was almost completely inhibited when the leaves were removed.

3. Intact and defoliated cuttings cultured in sucrose in darkness produced the complete response obtained in plants in the light. Proliferations and root primordia were formed in abundance. Similar results were obtained with maltose, levulose, glucose, and lactose; mannitol was not effective.

4. Cuttings were left in the dark for 1-6 days before treatment. Those maintained on a sucrose nutrient showed no differences due to pretreatment, but those in the mineral nutrient alone showed progressively less response through the third day and none at all beyond that.

5. Cuttings treated with lanolin paste for 3 days while in the mineral solution were then transferred to the sucrose medium. Six days later, when harvested, the treated regions had proliferated and produced root primordia. The response was not so great, however, as in cuttings cultured in sucrose but not treated until the time the minus-sucrose plants were put into the sugar medium.

6. Bean cuttings in a mineral nutrient in the dark also showed decreased response to the growth substance and complete response when cultured in sucrose.

7. Galactose was toxic to both tomato and bean cuttings.

8. Tomato cuttings in a minus-nitrogen medium responded as vigorously to the growth substances as did those with nitrogen. Nitrogen-deficient plants and cuttings also proliferated and gave rise to root primordia.

9. The most obvious cytological change in response to the growth substances in both the bean and tomato was the great increase in size of the nucleolus. Under conditions of carbohydrate deficiency the nucleolus diminished in size and finally disappeared. No changes occurred in the nucleoli of plants cultured in a sugar medium in the absence of growth substances.

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LITERATURE CITED

1. ALEXANDER, T. R., Carbohydrates of bean plants after treatment with indole-3-acetic acid. *Plant Physiol.* 13:845-858. 1938.
2. BAUSOR, S. C., REINHART, W. L., and TICE, G. A., Histological changes in tomato stems incident to treatment with β -naphthoxyacetic acid. *Amer. Jour. Bot.* 27:769-779. 1940.
3. BEAL, J. M., Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. *BOT. GAZ.* 102:366-372. 1940.
4. BONNER, D. M., HAAGEN-SMIT, A. J., and WENT, F. W., Leaf growth hormones. I. A bio-assay and source for leaf growth factors. *BOT. GAZ.* 101:128-144. 1939.
5. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. *BOT. GAZ.* 98:491-519. 1937.

6. COOPER, W. C., Hormones in relation to root formation on stem cuttings. *Plant Physiol.* 10:789-794. 1935.
7. ———, Transport of root-forming hormone in woody cuttings. *Plant Physiol.* 11:779-793. 1936.
8. ———, Hormones and root formation. *BOT. GAZ.* 99:599-614. 1938.
9. EATON, F. M., Interrelations in the effects of boron and indoleacetic acid on plant growth. *BOT. GAZ.* 101:700-705. 1940.
10. FLIRY, M., Zur Wirkung der Endknospe auf die Hypokotylstreckung des Dikotylenkeimlings. *Jahrb. wiss. Bot.* 77:150-184. 1933.
11. KNUDSON, L., Toxicity of galactose for certain of the higher plants. *Ann. Missouri Bot. Gard.* 2:659-666. 1915.
12. ———, The toxicity of galactose and mannose for green plants and the antagonistic action of other sugars toward these. *Amer. Jour. Bot.* 4:430-437. 1917.
13. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. *BOT. GAZ.* 101:204-225. 1939.
14. LOCKE, S. B., RIKER, A. J., and DUGGAR, B. M., Growth substances and development of crown gall. *Jour. Agr. Res.* 57:21-39. 1938.
15. MILLER, E. C., *Plant physiology*. New York. 1938.
16. MITCHELL, J. W., Effect of naphthalene acetic acid and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plants. *BOT. GAZ.* 101:688-699. 1940.
17. MITCHELL, J. W., and HAMNER, C. L., Stimulating effect of beta(3)indoleacetic acid on synthesis of solid matter by bean plants. *BOT. GAZ.* 99:569-583. 1938.
18. MITCHELL, J. W., KRAUS, E. J., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves following spraying with alpha naphthalene acetic acid emulsion. *BOT. GAZ.* 102:97-104. 1940.
19. MITCHELL, J. W., and MARTIN, W. E., Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants. *BOT. GAZ.* 99:171-183. 1937.
20. MITCHELL, J. W., and STUART, N. W., Growth and metabolism of bean cuttings subsequent to rooting with indoleacetic acid. *BOT. GAZ.* 100:627-650. 1939.
21. MITCHELL, J. W., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves as affected by application of growth-regulating substances. *BOT. GAZ.* 102:393-399. 1940.
22. NIGHTINGALE, G. T., The chemical composition of plants in relation to photoperiodic changes. *Univ. Wisconsin Agr. Exp. Sta. Bull.* 74. 1927.
23. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. *New Jersey Agr. Exp. Sta. Bull.* 461. 1928.
24. OLIVER, R. W., Honey as a stimulant to the rooting of cuttings. *Sci. Agr. (Ottawa)* 19:586-588. 1939.
25. ROBBINS, W. J., and SCHMIDT, M. B., Growth of excised tomato roots in a synthetic solution. *Bull. Torr. Bot. Club* 66:193-200. 1939.
26. SCHNEIDER, C. L., The interdependence of auxin and sugar for growth. *Amer. Jour. Bot.* 25:258-270. 1938.
27. SKOOG, F., Relationship between zinc and auxin in the growth of higher plants. *Amer. Jour. Bot.* 27:939-951. 1940.
28. SMITH, O., NASH, L. B., and DAVIS, G. E., Chemical and histological responses of bean plants grown at different levels of nutrition to indoleacetic acid. *BOT. GAZ.* 102:206-216. 1940.

29. STUART, N. W., Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. *BOT. GAZ.* 100:298-311. 1938.
30. STUART, N. W., and MARTH, P. C., Composition and rooting of American holly cuttings as affected by treatment with indolebutyric acid. *Proc. Amer. Soc. Hort. Sci.* 35:839-844. 1937.
31. SWEENEY, B. M., and THIMANN, K. V., Effect of auxins on protoplasmic streaming. II. *Jour. Gen. Physiol.* 21:439-461. 1938.
32. Went, F. W., Specific factors other than auxin affecting growth and root formation. *Plant Physiol.* 13:55-80. 1938.
33. ———, Effects of light on stem and leaf growth. *Amer. Jour. Bot.* 28:83-95. 1941.
34. WHITE, P. R., Glycine in the nutrition of excised tomato roots. *Plant Physiol.* 14:527-538. 1939.

EXTRACTION OF AUXIN FROM MAIZE, FROM SMUT TUMORS OF MAIZE, AND FROM *USTILAGO ZEAE*¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 541

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Introduction

This investigation was undertaken to test the hypothesis that if growth-affecting or growth-affecting substances play an important role in the normal growth of plants, then the growth disturbances observed in pathological conditions, such as in the corn-smut tumors being studied, should be correlated with disturbed auxone relations in the infected plants. Although many substances are classified as auxones, only those (the auxins) which can be detected by the standard *Avena* coleoptile technique have been studied here. It is not assumed, however, that auxins thus determined are the sole or primary causes of the responses incited by the pathogen, *Ustilago zeae*, but rather that they may be among the compounds correlated with the abnormal activity of the diseased tissues.

Early in this investigation it became apparent that the current procedures for the extraction of auxin from plant material were not adequate, for various reasons. Work done in this laboratory and elsewhere has shown that quantitative auxin extraction from green tissues is not easily accomplished (5, 10, 12, 20). As yet no adequate technique has been found that will remove all the auxin from tissues rapidly and with one extraction. Recently LINK, EGGERS, and MOULTON (10) described methods for the preparation of large, thoroughly homogeneous, desiccated samples from which sub-samples can be taken. Material thus prepared is highly suitable for auxin-extraction studies.

In this work an extensive study of the extraction and behavior of auxin from smut tumors of corn was undertaken. Also, for source material, corn stems, corn leaves, corn tassels, and four strains of the corn-smut fungus, *U. zeae*, were used to some extent. This investigation was continued over a period of 3 years, during the course of which about 3000 pans of *Avena* were used in the tests. Most of the experiments have been repeated, some of them many times.

Material and methods

A strain of Northwestern Dent corn and four strains of *Ustilago zeae* were obtained from Dr. E. C. Stakman of the University of Minnesota. The corn was grown in the greenhouse in garden soil, using 12-inch pots with four plants to each

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pot. The tumors were produced by inoculating corn plants when they were about 1 foot tall. Injections of *U. zae* were made into the region of the growing point with a hypodermic syringe. Monosporidial cultures of *U. zae*, designated 10I₁, 10K₂, 10J₃, and 10J₄, were grown on a liquid 0.1 per cent bacto-tryptone-dextrose medium. To incite tumor production, except for certain solopathogenic strains of the microorganism, it is necessary to mix + and - strains at the time of inoculation. Strains 10I₁ and 10K₂ yield only local lesions but no tumors, when injected into the plants separately; when mixed in liquid culture just before inoculation, the two together are highly pathogenic and large tumors result, located principally on the stems and leaves. These two strains were used exclusively to incite the tumors for extraction. Strains 10J₃ and 10J₄, being solopathogenic, are each capable, when injected into the growing point, of inciting tumor formation. They were not, however, as virulent as a mixture of + and - strains. Studies were made on the yields of auxins from the mats of the fungus grown on liquid media, and in this work all four strains were tested. About 3-4 weeks after inoculation, tumors appeared and continued to be produced over a period of 2 weeks. In the autumn of 1940, samples of normal leaves and of tumors from stems and leaves were collected, frozen immediately by crushed dry-ice (CO₂) mixed with the methyl ester of ethylene (cellosolve). This gives a temperature of -80° C. and freezes the material rapidly. While frozen, the tissues were dried *in vacuo*. The dried material was ground in a Wiley micro-mill to pass a 60 mesh and then stored in an evacuated desiccator over phosphoric pentoxide. The powders proved to be stable and yielded active extracts. The auxin was assayed by means of the standard *Avena* test (22), using an initial decapitation period of 2 hours. Photographs were taken 100 minutes after application of agar blocks.

The *Avena* tests were made in a room especially constructed for the purpose. The temperature was held at 24° C., the relative humidity at 88 per cent, and a continuous supply of fresh air was brought in and circulated by fans. For the test plants a strain of Swedish Victory oats sold by Vaughan's Seed Company was used. This seed gave very satisfactory results. Germination was high, the coleoptiles grew at a uniform rate, the leaves pulled easily, and the sensitivity was high. The coleoptiles were calibrated at each test with standard agar blocks containing indoleacetic acid, the concentrations being 0.015 and 0.030 mg. per liter of agar. (The average curvature obtained with the former concentration was about 12°.)

Three extractants were used for the auxin extractions: an aqueous ethyl ether, dry ethyl ether, and water. The aqueous ether was freshly distilled for each experiment over wet FeSO₄ and CaO. Dry ether was obtained by treating it with potassium permanganate, drying with calcium chloride, and distilling over sodium; it was stored over sodium in the dark in a refrigerator. Prepared by either method it was always found to be peroxide free. When ether was used as the extractant

the extracts were evaporated to dryness in a water-bath at 50° C. and taken up in 2 cc. of melted 1.5 per cent agar. Serial agar dilutions were then poured into an 8×10×1.5 mm. mold. After hardening, the agar cast was cut into twelve equal blocks, which were applied to the *Avena* coleoptiles. In each dilution tested, twelve coleoptiles were used.

When water was the extractant, the extract was not taken to dryness. Instead, 1 cc. was pipetted from the sample to be tested and mixed directly with 1 cc. of melted 3 per cent agar. The dilutions and blocks were prepared as before. In all the extractions, care was taken not to overheat the preparations, and when the extracts were removed from a vessel the latter was washed two or three times with ether. Whenever duplicate samples were extracted and assayed together, they each gave the same number of units of auxin, within the limits of the variability of the *Avena* test itself.

The results of the tests have been expressed in arbitrary units for convenience of comparison where different materials and different methods of extraction were used. The arbitrary activity unit is defined as that amount of substance which—when put in 1 cc. of 1.5 per cent agar—causes 1° curvature after 100 minutes in the *Avena* test.

Experimental results

ETHER EXTRACTION OF FRESH MATERIAL

EXTRACTS OF ZEA MAYS.—During this work, fresh leaf samples were collected from corn of various ages and extracted in the usual way. Leaves from young corn plants were invariably found to be low in auxin. The highest auxin yields were obtained from leaves of plants coming into tassel. The tassels themselves were exceedingly high in auxin.

EXTRACTS OF CORN-SMUT TUMORS.—A 50-gm. sample of fresh tumor material from leaves and stems was collected in July, 1940, and placed in peroxide-free wet ether in the refrigerator at 4° C. for extraction. Care was taken to collect only young and medium tumors, older sporulating ones being avoided. The tumors obtained in the greenhouse were white or yellow and contained very little chlorophyll. The tissues, almost intact, were placed in 50 cc. of extractant per gram of fresh material. After 12 hours, the ether was removed from the sample, and the sample was washed with fresh ether and put back in new ether for a second extraction. The ether taken off was evaporated to dryness and tested by mixing the extract with 2 cc. of melted 1.5 per cent agar. Serial dilutions were made from this and assayed in the *Avena* test. The results of a series of five extractions are given in table 1. It will be observed from the table that in the first extraction the *Avena* curvatures are small and there is a lack of proportionality between the dilutions and the curvatures. This lack of proportionality was always observed in the first

extraction of the fresh tissues of tumors. In later extractions this peculiarity is less evident. It seems likely that in the first extraction there are masking substances present which prevent the expression of the auxin. The nature of these substances is unknown. Previous work (4, 8, 18, 17, 16, 21) indicates that substances with masking or inhibitory effects are frequently found.

Table 1 shows that after five successive ether extractions the sample was still yielding auxin, perhaps indicating continued auxin production over a period of time by the tumors. More data bearing on this point are presented later. Extractions of tumors from ears of corn collected in the field gave results in agreement with those just reported.

TABLE 1
ACTIVITY OF ETHER EXTRACTS OF FRESH SMUT TUMORS OF
MAIZE EXPRESSED IN AVERAGE CURVATURE PER
12 AVENA COLEOPTILES (DEGREES)

AMOUNT TESTED (GM.)*	TESTING DATES				
	7/12/40	7/19/40	7/22/40	7/25/40	8/16/40
50.....	7.1	14.3	13.9	19.2	12.0
25.....	7.8	16.8	11.2	16.9	10.0
12.5.....	8.7	17.3	3.6	12.6	3.7
6.8.....	9.2	15.8	1.5	6.8	0
3.4.....	6.8	8.8	0	3.1
1.7.....	2.8	4.9	0

* Number of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5 per cent agar.

AUXIN CONTENT OF CORN LEAF SHEATH TUMORS COMPARED WITH NORMAL LEAF SHEATHS.—Fresh material was used and treated as already described. After 4 days of extraction the leaf sheath tumors gave forty-eight units of auxin as compared with three units for the normal sheaths. This relationship in which tumors are always higher in auxin than the normal tissues was found when the experiment was repeated and when repeated extractions of the same materials were made.

ETHER EXTRACTION OF FROZEN VACUUM-DRIED MATERIAL

AUXIN FROM SMUT TUMORS OF MAIZE: EXPERIMENTS WITH DRY ETHER.—Recently the development of the frozen vacuum-drying method for the treatment and storage of plant material has made it possible to compare different methods for the extraction of auxins. Using one finely ground sample of tumors of corn smut, studies were made of procedures for the extraction of auxin by drawing subsamples, treating them in various ways, and comparing the results of the different treatments.

With the dried material available for extraction, absolutely dry ether was prepared and used in making extractions. Dried material gave no activity when dry ether was the extractant. Independently of the present work, and using oven-dried material, THIMANN and SKOOG (20) have reported similar findings and concluded that water is necessary for auxin extraction of dry material. That auxin is soluble in dry ether has been demonstrated (10). Wet ether extracts of nodules of kidney bean, when dissolved in dry ether and then taken to dryness and assayed, showed the same activity as comparable wet ether extracts when taken up directly in agar. The conclusion is that water plays a part in the progressive liberation of auxin.

There was the possibility that the dried residue extracted by dry ether contained material which could be converted into auxin, if water were present. This was tested by a series of three extractions with dry ether, A, B, and C. Sample A

TABLE 2
ACTIVITY OF ETHER EXTRACTS OF 8 IDENTICAL 20-MG. SAMPLES
OF VACUUM-DRIED SMUT TUMORS FROM MAIZE

DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN	DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN
1/10/41.....	6	72	3/4/41.....	7	96
1/16/41.....	6	96	3/4/41.....	7	96
1/23/41.....	7	96	3/8/41.....	7	144
1/25/41.....	2	56	3/8/41.....	7	80

was tested without further treatment, B was treated with 1 cc. of water for 14 hours, and C with 1 cc. of water for 3 days. Extracts of A, B, and C were inactive in the *Avena* test, and it was concluded that dry ether does not remove any substance which can later be activated by the addition of water.

AUXIN FROM SMUT TUMORS OF MAIZE: EXPERIMENTS WITH WET ETHER.—Extractions of eight 20-mg. samples of frozen vacuum-dried material were made at different times with wet ether and are compared in table 2. The data are presented in terms of the arbitrary auxin units, defined earlier in the paper. Twenty cc. of ether was used for each sample and kept in the refrigerator at 4° C. The wet ether used in all the following experiments was prepared by shaking 15 cc. of dry ether, prepared according to the method already described, with 0.25 cc. of water. This amount of water saturated the ether. The procedure eliminated one source of variability in the extractions, since the dry ether used in all the extractions came from the same supply and contained the same amount of water. The ether used was in the ratio of 1000 cc. per gram of powder. The material remained in ether 7 days, except in two instances in which one extraction was for 2 days and the other 6 days. From four of the extracts 96 units were obtained (table 2); one gave

56 units, another 80, another 144, and another 72. When extracted for only 2 days, the auxin yield was 56 units. When the period of extraction in ether was the same, the number of units obtained was remarkably uniform. That time is a factor in the extraction process is shown in the following experiment.

Each of four 30-mg. samples of tumor powder, prepared according to the freezing desiccation method, was placed in 20 cc. of wet ether. Sample 1 was extracted one day; sample 2, two days; sample 3, three days; sample 4, seven days. The experiment was arranged so that the extractions were completed simultaneously, and all were assayed simultaneously. For sample 1 the yield was 44 units; for sample 2 it was 80 units; for sample 3 it was 120 units; and for sample 4 it was 320 units. There is good proportionality between time in wet ether and yield of auxin.

Tests to determine whether the amount of extractant used was a limiting factor showed that different quantities of ether resulted in practically the same number

TABLE 3
AUXIN FROM 20 MG. OF VACUUM-DRIED TUMORS FROM MAIZE SAMPLE
EXTRACTED WITH SUCCESSIVE 20-CC. VOLUMES OF ETHER

DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN	DATES OF TESTS	DAYS IN ETHER	UNITS OF AUXIN
3/31/41.....	3	48	5/18/41.....	10	68
4/18/41.....	18	384	5/29/41.....	11	104
4/23/41.....	5	112	6/7/41.....	10	66
5/8/41.....	15	176			
Total to date.....				72	1018

of units of auxin. This corroborates the former observation that auxin is gradually liberated from dried material in the presence of water.

Experiments with repeated extractions of material with wet ether give further support to this observation and are of interest in comparison with the repeated extractions of fresh material discussed earlier. From one series of repeated extractions (table 3) it is evident that the fresh and the dried material on extraction gave similar results. When extracted, both yielded auxin over a long period. Here again the yield of auxin is a function of the extraction time. The fresh material differed greatly from the dry powder, however, in that dry material yielded about 100 times as much auxin as the fresh when the same number of grams of each, on a fresh-weight basis, were used in making the extractions.

In the repeated extractions just described, either dry ether or wet ether was used alone. In another experiment, two 30-mg. samples, A and B, were first extracted with wet ether for 14 days and tested, the residues dried by washing with dry ether, followed by a second extraction in dry ether for 8 days, after which these extracts were tested while the residues were placed in wet ether for a third extrac-

tion. In this third extraction, sample A was in wet ether for 5 days and B for 12 hours. The first extraction with wet ether gave for A and B, 320 and 277 units, respectively; the second extraction with dry ether gave 0 units in each case; the final wet ether extracts were active. From sample A after 5 days, 138 units were obtained, while the yield from B after 12 hours was 40 units. This is further evidence of the part played by water and the importance of the time factor in auxin liberation.

The Soxhlet method of extraction with wet ether was tried with the possibility that auxin in the unbound form might be rapidly removed in this way. For the

TABLE 4

ACTIVITY UNITS OF EXTRACTS OF TWO 20-MG. SAMPLES OF POWDER SOXHLETIZED FOR 18 AND 47 HOURS, RESPECTIVELY. FOLLOWING THE 18-HOUR SOXHLETIZATION THIS SAMPLE WAS EXTRACTED BY STANDING IN SUCCESSIVE VOLUMES OF ETHER IN ICEBOX

DATE OF EXTRACTION	DAYS IN ETHER	SOXHLETIZED 18 HOURS	SOXHLETIZED 47 HOURS
3/21/41.....	288 (stand- ing)	384
3/22/41.....	1	14
3/28/41.....	6	28
4/12/41.....	16	60
4/22/41.....	10	32
5/7/41.....	15	28
5/29/41.....	21	12
6/7/41.....	10	9
Total.....	79	471

tumor material, this does not seem to be the case; almost all the auxin is bound. With short Soxhlet extractions of 3, 6, and 10 hours very low yields were obtained, scarcely greater than if the material had been merely standing in ether for the same periods. Table 4 summarizes the data from two Soxhlet runs, one of 18 hours' duration and the other of 47 hours. It is observed that there is no proportionality between the length of time the material was Soxhletized and the yield of auxin. Other materials extracted in the Soxhlet gave similar results, but no explanation for this behavior is offered.

After the 18-hour Soxhletization, the powder was re-extracted seven times with wet ether, over a period of 79 days. The total auxin yield, including the Soxhletization, was 471 units, as compared with an expected yield of about 1000 units for repeated extractions of the material when left standing in the refrigerator in wet ether. Considerable auxin was lost when the Soxhlet method was used.

Two Soxhlet extractions with dry ether, A and B, were followed by extraction

of A with wet ether and B with water (table 5). There was the possibility that when fats and other substances were removed by dry ether the process of liberation of auxin would be more complete and rapid. After this Soxhletization by dry ether, the material gave similar results in subsequent extractions to powders subjected to repeated wet ether extraction alone, although auxin yields were considerably lower after the Soxhlet procedure.

AUXIN FROM SMUT TUMORS OF MAIZE: WATER EXTRACTION. —A 20-mg. sample of tumor powder was extracted for 2 days in dry ether to which 0.5 cc. of water had been added. By separating the ether fraction from the water fraction and testing each separately for auxin content, it was determined that the water fraction was many times more active than the ether fraction. It seems that the extractable

TABLE 5
ACTIVITY UNITS OF EXTRACTS OF SAMPLES A AND B, EACH
SOXHLETIZED WITH DRY ETHER, A THEN EXTRACTED
BY STANDING IN WET ETHER 8 DAYS AND B IN WATER
8 DAYS. CONTROLS FOR A AND B EXTRACTED BY
STANDING IN WET ETHER AND WATER 8 DAYS IN ICE-
BOX, RESPECTIVELY

A WET ETHER		B WATER	
SOXHLETIZED, THEN IN ETHER 8 DAYS	CONTROL, IN ETHER 8 DAYS	SOXHLETIZED, THEN IN WATER 8 DAYS	CONTROL, IN WATER 8 DAYS
60	192	2176	2304

auxins are highly soluble in water and not very soluble in ether, indicating that the partition coefficient between ether and water is low for these auxins.

These findings suggested some studies on water extraction of the tumor powder. Some of the experiments are similar to those carried out with wet ether. They were devised so that the efficiency of ether and of water as extractants could be compared. Table 6 gives the results of a series of repeated extractions. Ten mg. of powder was put into 2 cc. of water for this series, the ratio of extractant to powder thus being 200 to 1. Experience had shown that more than enough water was present for the amount of powder to be extracted. In making the agar blocks for the *Avena* test, 1 cc. of water extract was put into 1 cc. of 3 per cent agar and dilutions made from this. The powder was put into previously unused distilled water for each subsequent extraction. All extractions were carried out in the refrigerator at about 4° C. Most of the auxin was liberated in the first three extractions. For example, 3008 units (table 8) were obtained from three extractions which

covered 22 days, and only 198 units were obtained in the next three extractions throughout a 33-day period. Comparable repeated extractions by ether and by water were made, and it was found that when water was the extractant the yield of auxin was greater than when ether was the extractant. The total number of units of auxin obtained by exhaustive extraction with water was 3000-3500, while with wet ether only 1000-1500 units were obtained. Greater yields of auxin were obtained in a given time when the material was extracted with water than when ether was the extractant. As an example, in one experiment the auxin yield was

TABLE 6
AUXIN FROM 10 MG. OF VACUUM-DRIED SMUT TUMORS OF MAIZE.
SUCCESSIVE WATER EXTRACTIONS EACH OF 2 CC.

DATES OF TESTS	DAYS IN WATER	UNITS OF AUXIN	DATES OF TESTS	DAYS IN WATER	UNITS OF AUXIN
4/21/41.....	9	1280	5/7/41.....	12	104
4/22/41.....	10	1152	5/18/41.....	11	88
4/25/41.....	3	576	5/28/41.....	10	6
Total.....				55	3206

TABLE 7
AUXIN FROM 5 MG. OF VACUUM-DRIED SMUT TUMORS
OF MAIZE. SUCCESSIVE WATER EXTRACTION EACH OF 2 CC.

TIME IN WATER	UNITS OF AUXIN	TIME IN WATER	UNITS OF AUXIN
$\frac{1}{2}$ hour.....	288	$\frac{1}{2}$ hour.....	0
$\frac{1}{2}$ hour.....	32	5 days.....	128

256 units in 2 hours, 512 in 24 hours, and 1536 in 7 days. In sharp contrast is the fact that wet ether gave on the average only 96 units in 7 days.

Another proof that auxin does not all come out at once when water is the extractant was provided by the following experiment. In this case 5 mg. of powder was put into 2 cc. of water for $\frac{1}{2}$ hour. The water was removed from the sample and the latter again put into 2 cc. of water. The material was treated thus with water three times for $\frac{1}{2}$ hour each time. Each 2 cc. of water removed was tested for auxins. Auxin was obtained in the first two extractions (table 7) but not in the third. The powder, apparently exhausted of auxin, was extracted again for 5 days. Tests showed 128 units present at the end of that time. Liberation of auxin must have occurred during the 5-day interval. The experiments were carried out at

2° C. and there was no growth of microorganisms. The experiment was repeated with similar results.

In the experiment (table 7) in which the powder was Soxhletized with dry ether and then extracted 8 days with water, it is noted that 2176 units were recovered, compared with 2304 units from control material not Soxhletized. Thus, removing fats and other substances with dry ether subsequent to water extraction did not aid the process of extraction.

Using water extracts, the effect of heat on the auxin extracted by water from smut tumors was studied. Ten mg. of powder was extracted in 4 cc. of water for 20 hours. At the end of this period, the water was divided into four aliquots. Sample 1 was placed in the icebox at 1.7° C., vials containing samples 2, 3, and 4 were

TABLE 8
EFFECT OF TEMPERATURE ON AUXIN STABILITY;
EXPOSURE 2 HOURS IN WATER

SAMPLE	TEMPERATURE (° C.)	UNITS OF AUXIN	SAMPLE	TEMPERATURE (° C.)	UNITS OF AUXIN
1.....	1.7	64	3.....	60	72
2.....	40	64	4.....	100	44

placed in hot-water baths at 40°, 60°, and 100° C., respectively, for 2 hours. The activity of the several extracts was then tested (table 8). The heat did not change the amount of auxin in the samples under the conditions of the experiment, except at 100° C.

ETHER EXTRACTION OF FOUR STRAINS OF *USTILAGO ZEAE*

Four strains of *U. zeae* were grown in a liquid bacto-tryptone medium. After 2 months the mats of the fungus were removed from the surface of the culture medium and placed in wet ether for extraction. The ether was then decanted off, the mats washed several times with fresh ether, and returned to fresh ether for a second extraction. The ether was taken to dryness after each of the two extractions, and the extracts were tested for auxins. It was found that almost all the auxins were removed in the first 24-hour extraction period. When third extractions were made no auxins were obtained. This is in contrast to green tissues which continue to yield auxins, even after ten or more extractions.

For another series of experiments the mats of the fungus were frozen and vacuum dried and the material then ground to a fine powder. The powders were treated with wet ether. Analyses showed the total auxin obtained to be greater than the total obtained by treating comparable fresh mats with ether. Experience has shown that no auxin is obtained when dry ether is used in the extraction of the dry powders.

Strains 10J₃, 10J₄, 10I₁, and 10K₂ were grown on two different media in order to determine whether or not different amounts of auxin could be obtained from the various strains grown on an organic bacto-tryptone medium, and also whether auxin could be obtained from mats grown on a synthetic medium containing inorganic salts and dextrose but no proteins or amino acids. The four strains were grown for two different periods (2 months and 9 months) on each of the two media. The mats were then frozen and vacuum dried. Ten-mg. samples of the strains of the fungus grown on the organic bacto-tryptone medium were extracted with 20 cc. of wet ether. Eighty-mg. samples of the fungus grown on the synthetic medium were extracted at the same time. Table 9 shows that extracts of the four

TABLE 9

ACTIVITY OF ETHER EXTRACTS FROM FOUR STRAINS OF *Ustilago zeae*. EACH STRAIN GROWN ON ORGANIC BACTO-TRYPTONE MEDIUM OR ON SYNTHETIC MEDIUM FREE OF PROTEINS AND AMINO ACIDS

STRAIN	BACTO-TRYPTONE MEDIUM		SYNTHETIC MEDIUM
	TWO MONTHS ON MEDIUM, UNITS OF AUXIN FROM 10-MG. SAMPLE	NINE MONTHS ON MEDIUM, UNITS OF AUXIN FROM 10-MG. SAMPLE	TWO MONTHS ON MEDIUM, UNITS OF AUXIN FROM 80-MG. SAMPLE
10J ₃	42	200	23
10J ₄	10	100	32
10I ₁	8	160	4
10K ₂	4	60	8

strains differ in the amount of auxin which can be obtained by extracting the powders. The extracts from the solopathogenic forms, except in one case, show a higher auxin content than the extracts from the non-solopathogenic ones. It can also be seen that auxin can be obtained from the fungus grown on the synthetic medium free of proteins and amino acids.

The powders from the fungus grown on the organic medium, however, yield about eight times as much auxin as the same amounts of powders from fungus grown on the synthetic medium. In the experiments just described the mats alone were tested for auxins. The next step was to test the liquid media that had supported the fungus.

Forty cc. of both types of liquid medium that had supported the mats of the fungus were dried separately *in vacuo*. The residues were dissolved in 2 cc. of water and mixed with 2 cc. of melted 3 per cent agar. The agar blocks for the *Avena* test were cast from this mixture. From both types of medium very active

extracts were obtained. They were about equally auxinic. The source of the auxin is not known. The organisms may synthesize auxin intracellularly, followed by its diffusion from the cells into the medium, or the cells may produce substances which diffuse into the medium and convert materials in the nutrient to auxin. The fungus on the bacto-tryptone may convert the tryptophane or other organic compounds (either intercellularly or intracellularly) into auxin. In the case of the fungus grown on the synthetic medium in which dextrose was the only organic substance, it seems likely that the auxins were produced intracellularly. The function of the auxins in the metabolism of the fungus, if they have any function, is not known. Perhaps they are katabolic products.

Discussion

Total auxin yields from frozen vacuum-dried powders from ground tumors of corn smut, or leaves, or the fungus *Ustilago zeae*, were higher than from the same objects in the fresh state. The same findings have been reported for kidney bean roots (10), for legume nodules, and for crown gall of tomato. THIMANN and SKOOG (20) noted that material which had been dried by heat gave a decreased yield of auxin, perhaps due to heating.

The results of this work, and of the investigations just cited, indicate that water plays some role in the liberation of auxin from plant tissues. That its action is hydrolytic has been suggested (10). SKOOG and THIMANN (15) have released bound auxin with proteolytic enzymes. Apparently any free auxin present in the living tissues is destroyed during the freezing and dehydrating process, or is fixed during these processes, since none is removed with dry ether—in which it is soluble.

The auxin from smut tumors is highly soluble in water, much more so than in ether, and comparisons of exhaustive extractions made with the two extractants indicate that the process is more rapidly and completely carried out with water. During the slow ether extraction, perhaps much auxin is destroyed by peroxides and otherwise, explaining the higher total yields from the more rapid water extraction.

All the auxin is obtained from *U. zeae* preparations in a single extraction with wet ether. This is in contrast to green tissues requiring ten or more extractions to obtain the same results.

Strains of *U. zeae* produced auxin when grown either on a bacto-tryptone medium or on a synthetic medium without proteins of amino acids. Auxins have been extracted from microorganisms grown on media containing amino acids or proteins (14, 7, 19, 1, 9, 2, 3). BURKHOLDER (2) demonstrated that *Aerobacter aerogenes* and *Escherichia coli* grown on glycerol-mineral salts-agar, in which the sole

source of nitrogen was either KNO_3 or NH_4Cl , produced auxin. GEORGI and BEGUIN (3) reported the elaboration of growth substances by four species of *Rhizobium* and *Bacillus radiobacter* when the culture medium contained tryptophane. They found, however, that when mannitol was the source of carbon and KNO_3 the sole source of nitrogen, no active substances were produced in the medium.

Solopathogenic strains of *U. zae* were found, with one exception, to produce more auxin than non-solopathogenic strains. This ability of the solopathogenic strains may be correlated with the capacity of the former to excite tumors in corn. LOCKE, RIKER, and DUGGAR (13) reported that for *Bacillus radiobacter* no consistent difference in the production of growth substances was observed between the virulent and the attenuated cultures. GEORGI and BEGUIN tested virulent and attenuated strains of *Rhizobium* and also found no correlation between pathogenicity and auxin yield.

HAAGEN-SMIT, LEECH, and BERGEN (6) have isolated crystalline indoleacetic acid from extracts of corn. This is the first time that this acid has been isolated from higher plants. These workers conclude that since the amount of the isolated acid was many times greater than the auxin amount indicated by the normal extraction process, it represented a large part of the auxin present in bound (precursor) form. Thus it is likely that the auxin extracts of corn contained indoleacetic acid and probably other active substances.

Differences in auxin content exist between smut tumors and similar regions of healthy tissues, the tumors being the more auxinic. LINK has found that in all cases where tumor material has been compared with healthy, the former was the more active. In this connection LINK and EGGERS (11) have recently reported on legume nodules and crown gall of tomato.

The higher activity of the tumors could be due to (a) the metabolic activity of the pathogen, or (b) the activity of the tumor tissues themselves in response to the injury of the host cells by the parasite. It is clear that auxin may be one of the causes of the pathological condition manifested by tumor production.

During the last 5 years many reports have appeared concerning the role of auxins in the physiology of plants. The only conclusion that can be reached from a review of the literature is that there is no simple relationship of growth-regulating substances to metabolism; apparently they play various roles, both direct and indirect. In general, auxins are found in highest concentrations in regions of the plant that are young and actively growing, that is, regions where the metabolic rate is high. It is possible that the presence of large amounts of auxins in the tumors studied is correlated with the increased and abnormal metabolism of the infected tissues.

Summary

1. The extraction of auxin by ether and by water from smut tumors of corn and other materials, frozen and vacuum dried, was studied. For a given weight of smut tumors, water extraction yields the greater amount of auxin. Dry ether extracts are not active. Water is necessary for the liberation of auxin from the tissues. Its action may be hydrolytic.

2. Smut tumors of corn yield auxin slowly with either water or ether extraction. The auxin is almost completely removed from the fungus, *Ustilago zeae*, in one ether extraction.

3. Smut tumors from corn leaves or stems yield more auxin than healthy leaves or stems.

4. Strains of *U. zeae* grown on a synthetic medium containing neither proteins nor amino acids are able to produce auxin.

5. Extracts of both types of medium upon which *U. zeae* had grown for 2 months contained much auxin and practically the same amount in each case.

6. The pathogenicity of strains of *U. zeae* seems to be correlated with their ability to produce auxin in a bacto-tryptone or a synthetic medium.

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LITERATURE CITED

1. BROWN, N. A., and GARDNER, F. E., Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. *Phytopath.* **26**:708-713. 1926.
2. BURKHOLDER, P. R., Production of growth substance by bacteria in media containing specific organic and inorganic nitrogenous compounds. *Amer. Jour. Bot.* **26**:422-428. 1939.
3. GEORGI, C. E., and BEGUIN, A. E., Heteroauxin production by efficient and inefficient strains of Rhizobia. *Nature* **143**:25. 1939.
4. GOODWIN, R. H., Evidence for the presence in certain ether extracts of substances partially masking the activity of auxin. *Amer. Jour. Bot.* **26**:130-135. 1939.
5. GUSTAFSON, F. G., Some difficulties encountered in the extraction of growth substances from plant tissues. *Science* **92**:266-267. 1940.
6. HAAGEN-SMIT, A. J., LEECH, W. D., and BERGEN, W. R., Estimation, isolation, and identification of auxins in plant material. *Science* **93**:624-625. 1941.
7. KÖGL, F., and KOSTERMANS, D. G. F. R., Heteroauxin als Stoffwechselprodukt niederer pflanzlicher Organismen. Isolierung aus Hefe. *Zeitschr. Physiol. Chemie.* **228**:113-121. 1934.
8. LARSEN, PAUL, Über Hemmung des Streckenwachstums durch natürlich vorkommende, Atherlösliche Stoffe. *Planta* **30**:160-167. 1939.

9. LINK, G. K. K., WILCOX, HAZEL W., and LINK, ADELINE D., Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumefaciens* extracts, β -indoleacetic acid, and wounding. BOT. GAZ. 98:816-867. 1937.
10. LINK, G. K. K., EGGERS, VIRGINIA, and MOULTON, J. E., Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs: its extraction with dry ether. BOT. GAZ. 102:590-601. 1941.
11. LINK, G. K. K., and EGGERS, VIRGINIA, Hyperauxiny in crown gall of tomato. BOT. GAZ. 103:87-106. 1941.
12. LINSER, H., Zur Methodik der Wuchsstoffbestimmung. II. Die Extraktion von Pflanzenmaterial. Planta 29:392-408. 1939.
13. LOCKE, S. B., RIKER, A. J., and DUGGAR, B. M., Growth substance and the development of crown gall. Jour. Agr. Res. 57:21-39. 1938.
14. NIELSEN, N., Untersuchungen über einen neuen wachstrumsregulierenden Stoff: Rhozopin. Jahrb. wiss. Bot. 73:125-191. 1930.
15. SKOOG, FOLKE, and THIMANN, K. V., Enzymatic liberation of auxin from plant tissues. Science 92:64. 1940.
16. SNOW, R., An inhibitor of growth extracted from pea leaves. Nature 144:906. 1939.
17. STEWART, W. S., A plant growth inhibitor and plant growth inhibition. BOT. GAZ. 101:91-108. 1939.
18. STEWART, W. S., BERGREN, W., and REDEMAN, E. E., A plant growth inhibitor. Science 89:185. 1939.
19. THIMANN, K. V., On the plant growth hormone produced by *Rhizopus solinus*. Jour. Biol. Chem. 109:279-291. 1935.
20. THIMANN, K. V., and SKOOG, FOLKE, The extraction of auxin from plant tissues. Amer. Jour. Bot. 27:951-960. 1940.
21. VOSS, H., Nachweis des inaktiven Wuchsstoffes, eines Wuchsstoff antagonisten und deren Wachstumsregulatorische Bedeutung. Planta 30:252-285. 1939.
22. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

CARBOHYDRATE NUTRITION OF RHIZOPUS SUINUS

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(WITH ONE FIGURE)

Introduction

Many investigations have been reported concerning the relative suitability of different carbohydrates in supporting the growth of fungi. In no case was attention paid to the purification of the sugars. Several observations on the effect of purification of a sugar upon growth of an organism upon that sugar make this question an important one where nutrition of the fungi is concerned.

Perhaps the earliest work relating to this question was that of WILDIERS (14). In apparently pure sucrose he found a water-soluble substance indispensable for the growth of yeast which could be extracted from the sucrose by 80 per cent alcohol. FUNK and FREEDMAN (5) determined that this substance could be removed from the sucrose by one recrystallization from 80 per cent alcohol. ALLISON and HOOVER (1) reported that one recrystallization of commercial sucrose from hot 70 per cent alcohol removed most of the substance in the sugar which was active as a growth factor for *Rhizobium trifolii*. Extractions of the sucrose in a Soxhlet extractor and evaporation of the extract to dryness gave a product which, although it was 75 per cent sugar, caused increases up to twenty-five fold in the growth of *R. trifolii*. HALL, JAMES, and STEWART (6) reported that recrystallization of sucrose from 80 per cent alcohol resulted in marked reduction in the yeast crop which could be grown by the use of this sugar. The ash obtained from pure sucrose caused no growth stimulation.

Few observations have been made with regard to the effect of the purification of the carbohydrate upon the growth of true fungi. SCHOPFER (11) found that a synthetic medium with purified maltose would not support growth of *Phycomyces* sp., but good growth resulted if Kahlbaum's maltose were substituted. According to SCHOPFER, it must be admitted that there is in the more impure sugar a substance whose role is all important in the development of *Phycomyces*. In the light of present knowledge of the growth relations of this fungus, it is evident that at least one substance present as an impurity in the sugars must have been vitamin B₁. STEINBERG (13) refluxed sucrose in a Soxhlet extractor with 95 per cent alcohol for 6 hours, drying the sugar at 103° C. to remove the alcohol. When *Aspergillus niger* was grown in a nutrient solution containing sucrose extracted in this manner, the dry weight production was reduced to 81 per cent of the original amount.

STEINBERG attributes this diminished growth to the removal of zinc and molybdenum. Similar results were obtained when heavy metals were omitted from the culture solution. STEINBERG believes that growth increases obtained with accessory growth materials are due, at least partially, to the presence of heavy metals in these substances.

Material and methods

Two methods of sugar extraction were used in these experiments. In the first method, 100 gm. of the sugar was stirred vigorously with 500 cc. of absolute alcohol. The mixture was filtered with suction on a Buchner funnel and washed with an additional 500 cc. of alcohol. The sugar was then removed from the funnel, placed in a large beaker, and stirred with 500 cc. of ether. This was also filtered and dried until there was no odor of ether remaining. In the second method, the method just described was preceded by extraction with 500 cc. of acetone, by filtration, and by washing with an additional 500 cc. of acetone. In subsequent discussion, these two methods will be referred to as the alcohol-ether method and the acetone-alcohol-ether method, respectively.

Liquid cultures were employed in these experiments. The medium consisted of various carbon sources used in different concentrations. The mineral nutrient part of the culture solution consisted of:

Ammonium nitrate.....	0.1M
Potassium dihydrogen phosphate.....	0.05M
Magnesium sulphate.....	0.01M
Ferric tartrate.....	trace

Fifty-cc. portions of the medium were placed in 150-cc. Erlenmeyer flasks and sterilized in an autoclave at 15 pounds' pressure for 20 minutes. Each flask was inoculated with 0.5 cc. of a spore suspension prepared by washing the surface of an agar slant culture with Ringer's solution. Enough of the spore suspension was prepared each time so that an entire series could be inoculated from the same suspension. After 8 days, the mycelium was placed on a previously dried and weighed filter paper, rinsed several times with distilled water, and dried in a hot-air oven at 95° C. for 48 hours. The dried mats were removed from the oven to a desiccator, cooled, and weighed rapidly on a chainomatic analytical balance. The fungus used, *Rhizopus suinus* Niels., was isolated by CHRISTIANSEN (3) and described by NIELSEN (9).

Experimentation

Table 1 gives the data for the first series of cultures with the extracted dextrose. It may be seen that extraction of dextrose with alcohol and ether results in the removal from the sugar of a substance or substances which are in some manner growth inhibiting. Further, a preliminary extraction with acetone removes an

additional amount of the substance or substances, or some other substance. These results were confirmed in a second series of cultures, this time involving only two different concentrations of dextrose. Each figure for the average mat weight in table 2 is the average obtained from twenty-four cultures.

The data in table 3 and figure 1 show that extraction of the galactose and dextrose causes considerable increase in the weight of the mycelium of *R. suinus*

TABLE 1
EFFECT OF ALCOHOL-ETHER AND ACETONE-ALCOHOL-ETHER EXTRACTIONS
OF DEXTROSE ON GROWTH OF RHIZOPUS SUINUS

CONCENTRATION OF DEXTROSE	AVERAGE MAT WEIGHT (MG.)			PERCENTAGE OF CONTROL	
	UNEXTRACTED	ALCOHOL- ETHER EX- TRACTED	+ ACETONE TREATMENT	ALCOHOL- ETHER TREATED	ACETONE- ALCOHOL- ETHER TREATED
0.005M.....	18	19	18	106	100
0.01M.....	25	26	28	104	112
0.02M.....	39	44	42	113	108
0.03M.....	49	52	58	106	118
0.05M.....	56	63	73	113	130
0.10M.....	61	68	106	112	174
0.20M.....	79	98	138	124	174

TABLE 2
EFFECT OF EXTRACTION OF DEXTROSE ON GROWTH OF RHIZOPUS SUINUS

CONCENTRATION OF DEXTROSE	AVERAGE MAT WEIGHT (MG.)			PERCENTAGE OF CONTROL	
	UNTREATED	ALCOHOL- ETHER	ACETONE- ALCOHOL- ETHER	ALCOHOL- ETHER	ACETONE- ALCOHOL- ETHER
0.02M.....	50	55	56	110	112
0.20M.....	89	124	138	140	156

which develops on the culture medium containing these sugars. In the extracted as well as in the unextracted state, galactose appears to be definitely superior to dextrose as a source of carbon for this fungus. HERR (7), EDGEcombe (4), and STEINBERG (12), however, state that galactose is a poor source of carbon for fungi.

Table 4 combines the results of several culture series. Only three concentrations of each sugar were used in these experiments. Enough of each sugar was extracted to provide sufficient material for the experiments cited in the table. Each figure for the mat weight is the average for twelve individual cultures.

TABLE 3

EFFECT OF EXTRACTION OF DEXTROSE AND GALACTOSE ON RELATIVE GROWTH OF RHIZOPUS SUINUS ON THE TWO SUGARS

CONCENTRATION OF SUGAR	AVERAGE MAT WEIGHT (MG.)				TREATED DEXTROSE AS PERCENTAGE OF UN-TREATED	TREATED GALACTOSE AS PERCENTAGE OF UN-TREATED
	UN-TREATED DEXTROSE	TREATED DEXTROSE	UN-TREATED GALACTOSE	TREATED GALACTOSE		
0.02M.	43	43	43	60	100	140
0.03M.	59	61	60	75	103	125
0.04M.	64	72	72	96	113	133
0.05M.	67	82	88	119	122	135
0.10M.	77	104	99	166	135	136
0.15M.	82	124	112	182	151	162
0.20M.	85	135	126	197	159	156

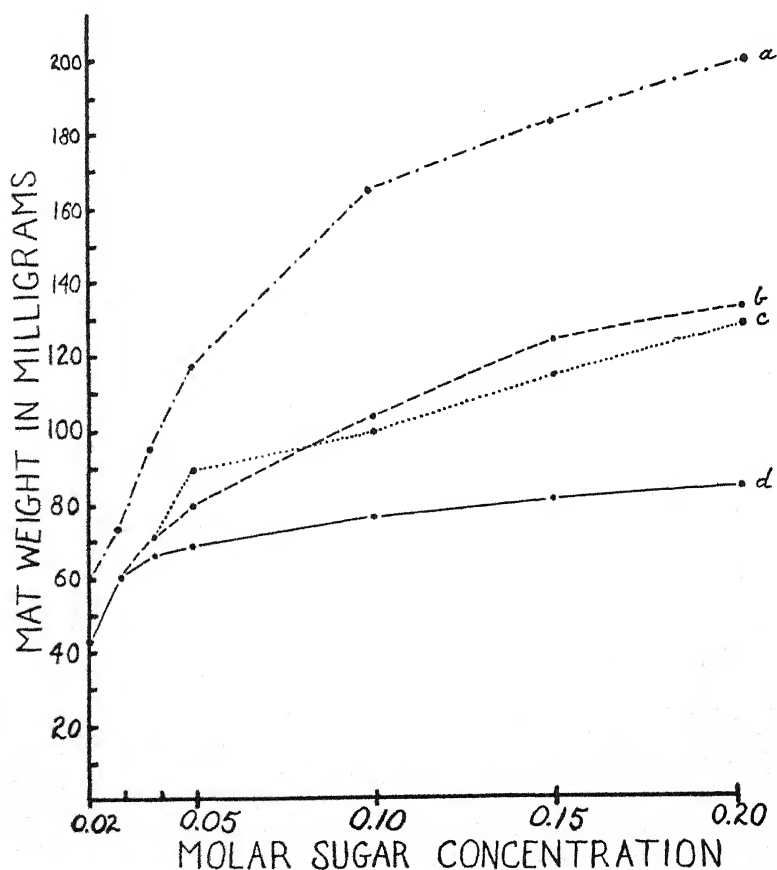


FIG. 1.—Comparison of growth of *Rhizopus suinus* on treated and untreated dextrose and galactose: a, treated galactose; b, treated dextrose; c, untreated galactose; d, untreated dextrose.

In all the cases studied, extraction of the sugars resulted in increased growth of *R. suinus* on the extracted sugar. The greatest increase was with levulose, where

TABLE 4
EFFECT OF EXTRACTION OF DEXTROSE, SUCROSE, AND LEVULOSE
ON GROWTH OF RHIZOPUS SUINUS ON THESE SUGARS

SUGAR	NO. OF SERIES*	CONCENTRATION	AVERAGE MAT WEIGHT (MG.)		PERCENTAGE UN-TREATED (CONTROL)
			UN-TREATED	TREATED	
Dextrose	1.....	0.05M	124	141	114
	2.....	0.05	96	107	113
	3†.....	0.05	78	90	116
	4.....	0.05	72	82	114
	5.....	0.05	84	94	112
	1.....	0.10	156	173	111
	2.....	0.10	113	132	117
	3†.....	0.10	103	118	115
	4.....	0.10	98	116	118
	5.....	0.10	118	139	118
	1.....	0.20	172	192	112
	2.....	0.20	131	148	112
	3†.....	0.20	135	148	110
	4.....	0.20	121	135	111
	5.....	0.20	140	160	110
Sucrose	1.....	0.05	15	17	114
	2.....	0.05	12	13	111
	1.....	0.10	40	47	118
	2.....	0.10	34	41	120
	1.....	0.20	80	92	115
	2.....	0.20	87	96	111
Levulose	1.....	0.05	73	103	142
	2.....	0.05	67	78	115
	1.....	0.10	99	127	128
	2.....	0.10	99	115	116
	1.....	0.20	149	188	126
	2.....	0.20M	131	161	123

* Individual series with corresponding numbers made at same time and inoculated from same spore suspension.

† Recrystallized from 80% alcohol, rather than extracted.

the average increase over the control was 22–28.5 per cent. Judging from its appearance, levulose was the most impure of the sugars.

The sugar used in series 3 was purified differently from that in the other four series. The dextrose was dissolved in as small a volume of hot water as possible,

and enough 95 per cent alcohol was added to make the final concentration 80 per cent. The solution was then stored in a cold place for 24 hours, during which time crystallization of the sugar took place. The supernatant alcohol was discarded, and the dextrose was dried. The results were not appreciably different from those obtained by extraction with the various solvents.

Since extraction or recrystallization of dextrose and other sugars resulted in an increased growth of *R. suinus* in the culture series in which the treated sugar served as the carbon source, it is evident that in the extract there must be a substance or complex of substances that retard assimilation of the sugar. Several attempts were made to determine the nature of this material. The first attempt was to obtain the extracted substance in a concentrated form. The extracts from 100 gm. of dextrose (1000 cc. acetone, 1000 cc. alcohol, and 500 cc. ether) were

TABLE 5
EFFECT OF ADDITION OF VARIED AMOUNTS OF
DEXTROSE EXTRACT TO CULTURE MEDIUM
UPON GROWTH OF RHIZOPUS SUINUS

AMOUNT OF EXTRACT (CC.)	AVERAGE MAT WEIGHT (MG.)	PERCENT- AGE OF CONTROL	AMOUNT OF SPORE FOR- MATION
0.....	74.6	100	Slight
1.....	105.0	142	Slight
3.....	139.8	187	Moderate
5.....	153.8	206	Heavy

evaporated to a small volume (100 cc. each) over a steambath and stored in a cold place until a large part of the dissolved sugar had crystallized. The three supernatant liquids were then combined and evaporated to dryness over the steambath. The dry residue was made up to 100 cc. in distilled water, so that each cubic centimeter of the extract contained the substances derived from 1 gm. of the sugar. A culture series containing 0.05*M* extracted dextrose, with from none to 5 cc. of the extract added, was inoculated with a spore suspension of *R. suinus*. Ten replicates of each concentration of the extract were used. The results are shown in table 5.

From the behavior of the fungus with respect to spore formation when different amounts of the extract were added to the culture medium, it was thought possible that at least one of the substances present in the extract was vitamin B₁ (thiamin). This supposition was based upon the behavior of agar plate cultures of *R. suinus* when synthetic vitamin B₁ was added to the medium. This work is to be discussed in detail in a forthcoming paper.

BARGER, BERGEL, and TODD (2) and JANSEN (8), among others, have shown that cautious oxidation of vitamin B₁ yields a yellow pigment, thiochrome, which shows

a blue fluorescence under ultraviolet light. A qualitative method for the determination of the presence of vitamin B₁ was used as follows, based on the method of BARGER *et al.*, for the formation of thiochrome.

To a solution of vitamin B₁ hydrochloride in 1-2 cc. of alcohol is added 2 cc. of 15 per cent potassium hydroxide in methyl alcohol and 1 cc. of an aqueous potassium ferricyanide solution. To this mixture is added 10 cc. of butyl alcohol, and the whole is shaken for about 2 minutes. The yellow pigment is separated in the butyl-alcohol layer and may then be tested for ultraviolet fluorescence.

Using synthetic vitamin B₁ in ethyl-alcohol solution, a fairly strong fluorescence was observed when 1 cc. of a 0.01 per cent solution of thiamin (0.1 mg.) was used. The oxidation was tried on the water solution of the dextrose extract, but the results were doubtful, since any fluorescence present was weak, and at the time of this particular experiment, suitable filters were not available.

The original methods of BARGER *et al.* and of JANSEN call for the use of a methyl-alcohol solution of the vitamin or of the material suspected to contain the vitamin. To obtain a critical test for the presence of vitamin B₁ in the sugar, a 100-gm. sample of dextrose was treated for several hours in a Soxhlet extractor with absolute methyl alcohol. The extract was then evaporated over a steambath to about 25 cc. and allowed to cool. A large part of the sugar dissolved by the extraction process crystallized out. The supernatant liquid was further concentrated to 10 cc.

Oxidation of the extract with alkaline potassium ferricyanide was carried out as already described. The resulting butyl-alcohol layer showed a yellow coloration, so that it was necessary to confirm the results by demonstration of ultraviolet fluorescence. The source of the ultraviolet was an 85-watt quartz mercury vapor lamp. In the opening in the lamp housing was placed a Corning 597 filter for transmitting the ultraviolet. When a Corning Noviol-A filter was superimposed upon the 597 filter, practically no light was transmitted. When the two filters were separated and the tube containing the yellow-colored butyl-alcohol layer was placed between them, a distinct blue color was observed through the Noviol filter, demonstrating the presence of thiochrome. Vitamin B₁ is therefore present in the reagent grade dextrose.

ROBBINS and KAVANAGH (10) showed that *Rhizopus nigricans*, both the plus and minus strains, grew satisfactorily in a liquid medium of mineral salts, asparagine, and dextrose, but its growth was materially reduced by the addition of 1 p.p.m. of vitamin B₁. SCHOPFER (11) had previously shown that various *Rhizopus* species, including *R. suinus*, showed reduction of growth when small amounts of vitamin B₁ were added to the culture medium. This line of evidence is consistent with the present finding that extraction of the sugars in the manner described (with consequent removal of traces of thiamin) results in increased growth of the fungus on the extracted sugar. In table 5, however, it may be seen that growth of

the fungus is roughly proportional to the amount of the concentrated extract added to the culture medium. A further extensive culture series was set up in order to confirm these rather striking results. A second dextrose extract was prepared and evaporated to dryness. To be sure that the increased vegetative growth upon addition of the extract was not a result of the dextrose contained in the extract, two precautions were taken. The sugar concentration of the medium was increased to 0.10*M*, and sets of cultures were used—each having an amount of 1.25 per cent dextrose solution added equal to the amounts of the dextrose extract used. The weight of the evaporated dextrose extract was 1.25 gm., and this was made up to 100 cc. with distilled water. Culture flasks were also prepared in which the culture

TABLE 6
EFFECT OF DEXTROSE EXTRACT AND OF VITAMIN B₁ ON GROWTH
OF RHIZOPUS SUINUS ON DEXTROSE

TREATMENT	AVERAGE MAT WEIGHT (MG.)	PERCENTAGE OF CONTROL	PERCENTAGE OF CULTURES WITH 1.25% DEXTROSE SOLUTION ADDED	SPORE FORMATION
Extracted dextrose.....	77.5	100	Weak
+1 cc. extract.....	123.0	160	153	Weak
+3 cc. extract.....	195.4	252	202	Moderate
+5 cc. extract.....	219.9	284	227	Heavy
+1 p.p.b. thiamin.....	69.5	90	Moderate
+1 p.p.m. thiamin.....	56.9	73	Moderate
+1 cc. 1.25% dextrose...	80.6	104	Weak
+3 cc. 1.25% dextrose...	96.5	125	Weak
+5 cc. 1.25% dextrose...	96.7	125	Weak

medium contained 1 p.p.m. and 1 p.p.b. of vitamin B₁. The results of this experiment are shown in table 6. The mat weight figures are the averages of ten cultures.

The concentrated extract of dextrose is shown to have great influence on vegetative growth and spore formation. Vitamin B₁ has a mild growth-retarding effect and a strong favorable influence on the formation of spores. Finally, in the cultures to which the 1.25 per cent sugar solutions were added, only a small part of the growth increase caused by the dextrose extract could be a result of the actual dextrose present in the extract. In an attempt to learn something of the nature of the growth-promoting material in the dextrose extract, the following procedure was adopted:

One hundred gm. of dextrose was ashed. The small amount of ash was dissolved in a small amount of hydrochloric acid, neutralized with sodium hydroxide with phenolphthalein as the indicator, and made up to 100 cc. with distilled water.

A culture series was set up, with 0.10*M* dextrose as the carbon source, and different amounts of the solution of ash elements were added to the medium. Ten replicates of each concentration were used.

Table 7 indicates that there are present in reagent-grade dextrose nonvolatile constituents (heavy metals) whose presence results in increased dry weight production by *R. suinus*. Possibly the growth relations of this fungus upon dextrose may be summarized in the following manner:

Reagent-grade dextrose of the brand used in these experiments contains traces of vitamin B₁ and small amounts of ash elements. The demonstration of other possible organic impurities is a matter for future consideration. The mineral elements cause increased growth of *R. suinus*, while vitamin B₁ causes decreased growth and

TABLE 7
EFFECT OF ADDITION OF DEXTROSE TO CULTURE
MEDIUM UPON GROWTH OF RHIZOPUS
SUINUS ON DEXTROSE

AMOUNT OF ASH SOLUTION ADDED	AVERAGE MAT WEIGHT (MG.)	PERCENTAGE OF UN- TREATED CONTROL	SPORE FORMATION
0.....	100	100	Slight
1.....	144	144	Slight
3.....	160	160	Slight
5.....	198	198	Slight

increased spore formation. It is suggested that the increase in growth on the dextrose which has been extracted with acetone, alcohol, and ether may be explained on the basis of the following facts. Removal of the vitamin B₁ eliminates the growth-retarding effect which has been demonstrated by ROBBINS and KAVANAGH, SCHOPFER, and the writer. This removal tends to cause increase in growth. The absence of spore formation would mean that a certain amount of energy and material, normally utilized in the production of spores, could be utilized in vegetative growth. This would counteract the unfavorable effect of the extraction process on growth of the fungus resulting from removal of heavy metals from the sugar. Concentration of the active agents which follows with concentration of the dextrose extract probably influences the effect of the heavy metals more than the effect of the vitamin B₁. Thus, when the concentrated extract is added to a culture, the effect of the heavy metals present more than balances the effect of the thiamin, and the result is augmentation of growth. Possibly the growth-retarding effect of vitamin B₁ is a corollary of the tendency of the vitamin to predispose the fungus to spore formation.

Summary

1. Extraction of dextrose, levulose, galactose, and sucrose with acetone, alcohol, and ether resulted in increased mycelial production by *Rhizopus suinus*.
2. Extraction of dextrose with alcohol and ether resulted in increased mycelial production by this fungus. The increase was not so great as with the acetone-alcohol-ether extraction.
3. Recrystallization of dextrose from 80 per cent alcohol resulted in growth augmentation of about the same magnitude as that which resulted from the acetone-alcohol-ether treatment.
4. The presence of vitamin B₁ (thiamin, aneurin) in reagent-grade dextrose was shown by the thiochrome oxidation method. Removal of traces of vitamin B₁ from the sugars during the extraction process is possibly the cause of the growth phenomena.
5. The extract of dextrose contains a substance which, when concentrated by evaporation, promotes the vegetative growth of *R. suinus* if it is added to the culture medium. The substance was demonstrated, by growth experiments in which the nutrient solution was supplemented with the ash obtained from the unpurified dextrose, to be an ash constituent.
6. Under the conditions of the extraction, the removal of vitamin B₁ would be more extensive than the removal of the ash constituents, the influence of the removal of the thiamin consequently being more effective in increasing the growth of *R. suinus* than the removal of the small amount of ash constituents in decreasing the growth of the organism. When the extract is concentrated, however, the influence of the ash element(s) on mycelial production is much greater than the influence of the vitamin B₁, and the result is growth augmentation.

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LITERATURE CITED

1. ALLISON, F. E., and HOOVER, S. R., An accessory factor for legume nodule bacteria. Sources and activity. Jour. Bact. 27:561-581. 1934.
2. BARGER, G., BERGEL, F., and TODD, A. R., Ueber das Thiochrom aus Vitamin B₁ (Aneurin). Ber. Deutsch. Chem. Ges. 68:2257-2262. 1935.
3. CHRISTIANSEN, M., Mucormykose beim Schwein. I. Mitt. Virchow's Arch. Path. Anat. Phys. 273:829-858. 1929.
4. EDGECOMBE, A. E., The effect of galactose on the growth of certain fungi. Mycologia 30: 601-624. 1938.

5. FUNK, C., and FREEDMAN, L., The presence of a yeast growth promoting vitamine in cane sugar. Jour. Biol. Chem. 56:351-860. 1923.
6. HALL, H. H., JAMES, L. H., and STEWART, L. S., Yeast growth stimulants in white sugars. Indust. Eng. Chem. 25:1052-1054. 1933.
7. HERR, W. H., Utilization of galactose by *Aspergillus niger* and *Penicillium glaucum*. Plant Physiol. 11:81-99. 1936.
8. JANSEN, B. C. P., A chemical determination of aneurin (vitamin B₁) by the thiochrome reaction. Rec. Trav. Chim. Pays-bas. 55:1046-1052. 1936.
9. NIELSEN, N., Mucormykose beim Schwein. II. Mitt. Beschreibung der isolierten Pilze. Virchow's Arch. Path. Anat. Phys. 273:859-863. 1929.
10. ROBBINS, W. J., and KAVANAGH, F., Vitamin B₁ or its intermediates and the growth of certain fungi. Amer. Jour. Bot. 25:229-236. 1938.
11. SCHOPFER, W. H., Etude de l'action du saccharose à divers états de pureté. Ann. Mycol. 33:28-32. 1935.
12. STEINBERG, R. A., Growth of fungi in synthetic nutrient solutions. Bot. Rev. 5:1-44. 1939.
13. ———, Relation of accessory growth substances to heavy metals, including molybdenum, in the nutrition of *Aspergillus niger*. Jour. Agr. Res. 52:439-448. 1936.
14. WILDIERS, E., Nouvelle substance indispensable au développement de la levure. La Cellule 18:313-336. 1901.

FOREST SEQUENCES IN THE NORTH CENTRAL STATES

PAUL B. SEARS

(WITH SIX FIGURES)

Introduction

This paper presents fifteen pollen profiles from as many peat deposits in Illinois, Indiana, Michigan, and Ohio, only two of which—Bucyrus and Mud Lake (Ohio)—have been previously published. These profiles have been prepared to show only the relative fluctuations of eight forest genera and one family, Betulaceae.

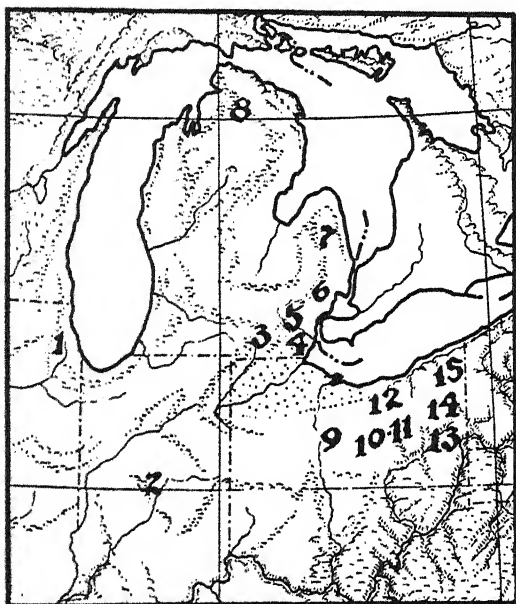


FIG. 1.—Location of bogs studied

The location of these deposits is indicated on the map (fig. 1) and again on each legend (figs. 2-6). Depth of samples analyzed is indicated by solid bars in each column, and by depth scale at the left. Length of bars represents relative percentages; but for more graphic comparison, bars are centered on scale instead of starting from zero point. In each graph, Roman numerals indicate corresponding stages.

A number of the profiles have been truncated by fire and cultivation. Others have been condensed because of drainage. Sedimentation began much earlier in some than in others, as is evident by noting positions relative to glacial retreat.

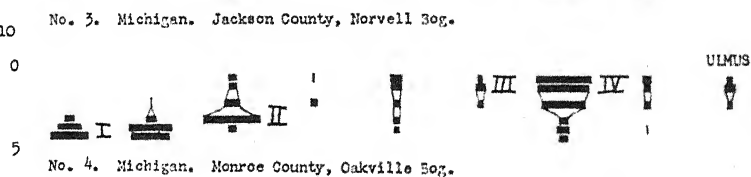
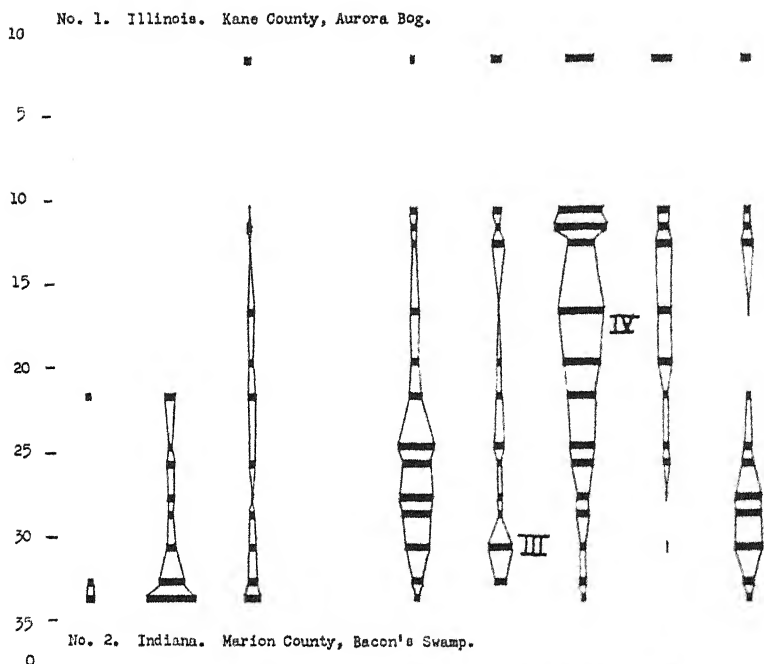
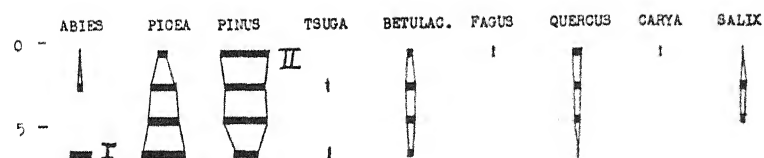
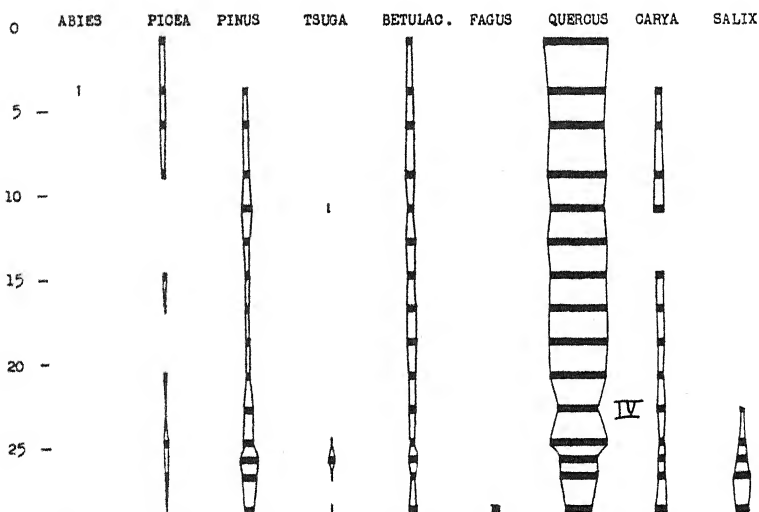
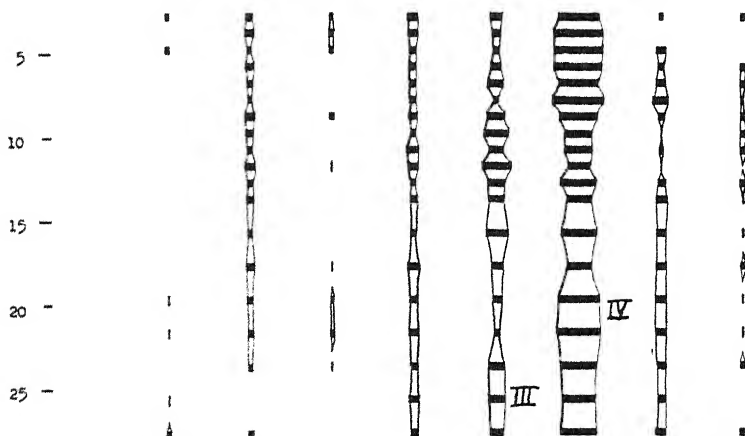


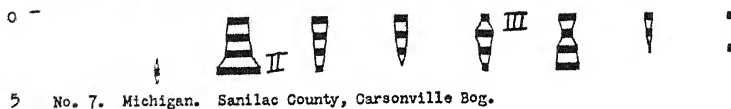
FIG. 2.—Pollen profiles of important forest components, bogs 1-4



No. 5. Michigan. Washtenaw County, Mud Lake Bog.

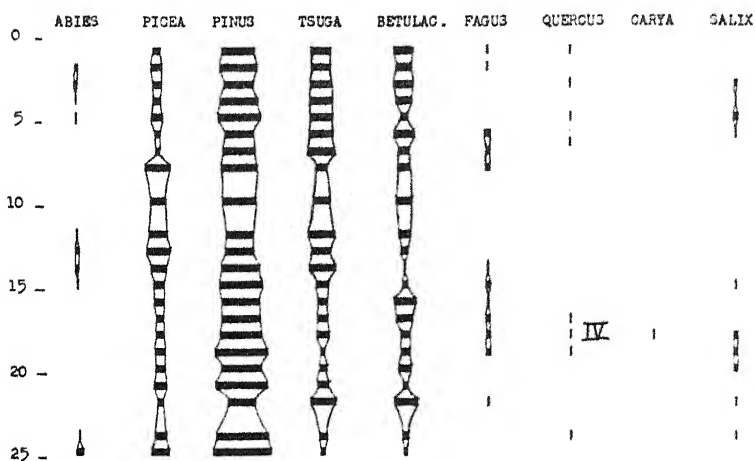


No. 6. Michigan. Macomb County, Mt. Vernon Bog.



No. 7. Michigan. Sanilac County, Carsonville Bog.

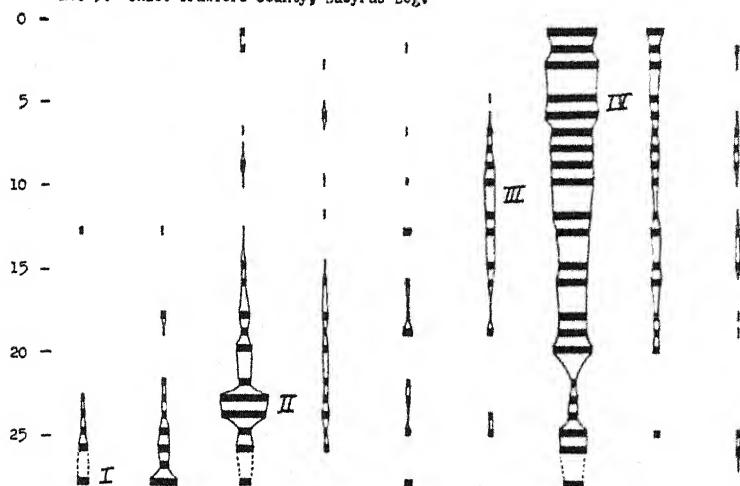
FIG. 3.—Pollen profiles of important forest components, bogs 5-7



No. 8. Michigan. Cheboygan County, Mud Lake Bog.



No. 9. Ohio. Crawford County, Bucyrus Bog.



No. 10. Ohio. Ashland County, Long Lake Bog.

FIG. 4.—Pollen profiles of important forest components, bogs 8-10

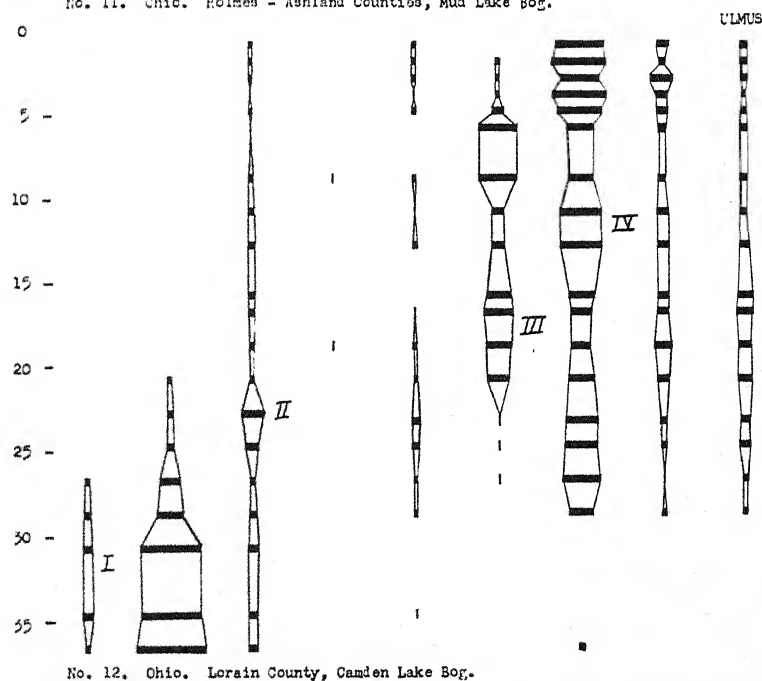
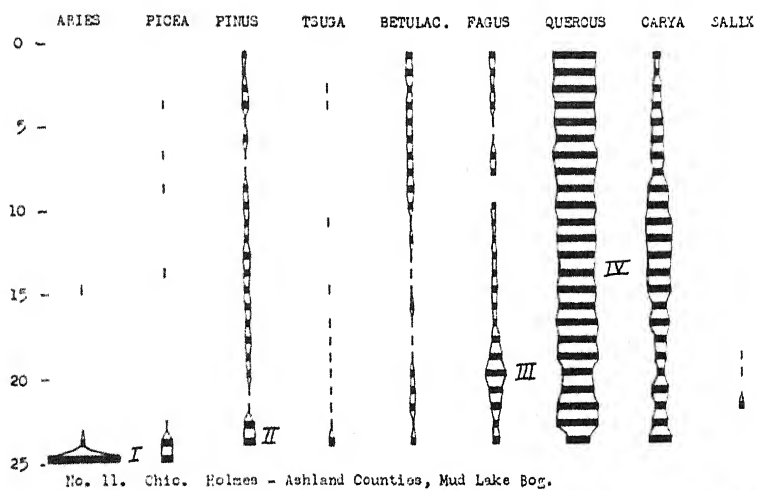


FIG. 5.—Pollen profiles of important forest components, bogs 11 and 12

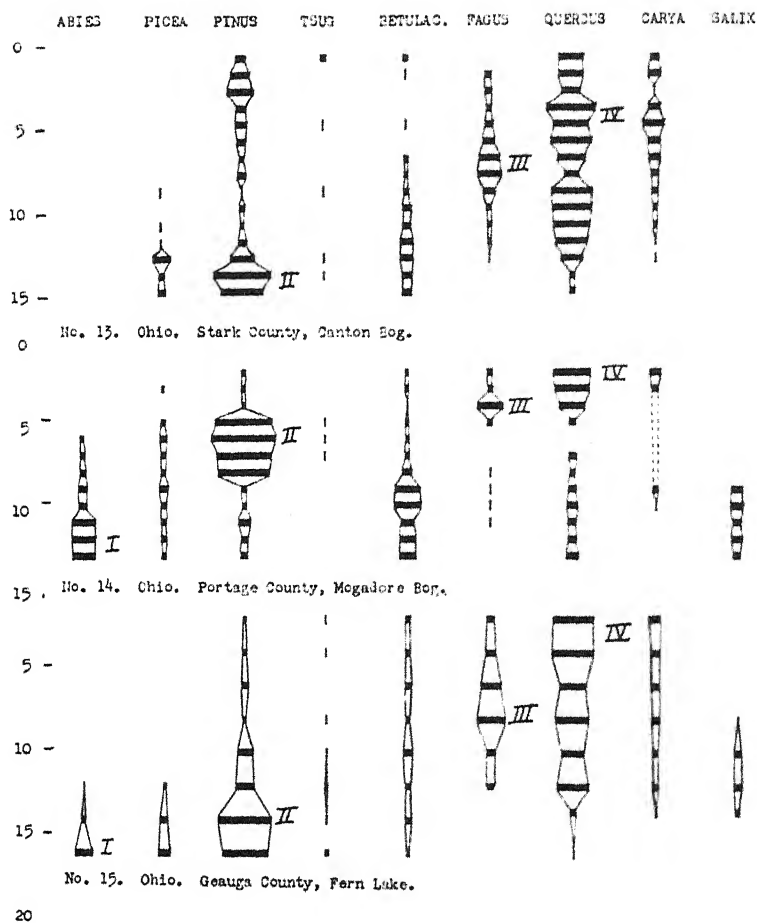


FIG. 6.—Pollen profiles of important forest components, bogs 13-15

Observations

1. AURORA BOG.—This profile was secured from material in which mastodon remains were found. It represents an early segment of postglacial time and shows a striking increase of pine at the expense of fir and spruce.

2. BACON'S SWAMP.—This profile shows a brief maximum of beech following spruce and pine. Beech gives way to birch-oak, followed by oak-hickory.

3. NORVELL BOG.—This profile shows pine reaching a maximum, then beech, followed by oak-hickory. The top of this deposit has been destroyed.

4. OAKVILLE BOG.—This is an old deposit, truncated and condensed. The shift from fir and spruce to a sharp pine maximum is evident. The next shift, from pine to oak, is marked by a faint interval during which beech is at a maximum, although never abundant. Elm behaves like beech.

5. MUD LAKE (WASHTENAW) BOG.—This is essentially post-coniferous. The presence of beech and hemlock at the bottom, with oak minimum, probably corresponds to the end of early beech maximum noted elsewhere.

6. MT. VERNON BOG.—This is likewise post-coniferous. Early and later beech shows maxima, inversely correlated with oak and hickory.

7. CARSONVILLE BOG.—This condensed deposit has been severely burned and drained. It begins with pine and oak maxima, followed by a beech maximum which is temporary. Beech yields to oak and hickory; but pine, birch, and hemlock remain as important constituents.

8. MUD LAKE (CHEBOYGAN) BOG.—This corresponds to a deciduous period farther south. Spruce, pine, hemlock, and birch are found throughout. Temporary displacement of hickory, oak, and beech by spruce and hemlock is notable, near the middle of the profile. In position this corresponds to the second (upper) beech maximum shown in certain other profiles. It suggests cooling.

9. BUCYRUS BOG.—This has been previously published (5). It shows in striking fashion the shift from fir and spruce to pine evident in the other early profiles of this report.

10. LONG LAKE BOG.—Here is a sharply marked pine maximum, following the waning of fir and spruce, and some distance above it a definite maximum of beech, replaced by oak and hickory toward the top.

11. MUD LAKE (OHIO) BOG.—This has been previously published (6). At the base is a telescoped record of shift from fir and spruce to pine, oak, and hickory. Above this is a brief but striking maximum of beech, giving way to an equally striking but more prolonged dominance of oak and hickory. In the original report the waning of hickory near the top and the increase of other species, not here included, was interpreted as an increase of mesophytism in recent times. In view of

the other profiles here presented, however, it may be questioned whether this was justified.

12. CAMDEN LAKE BOG.—Here the pine maximum, marked by disappearance of fir and waning of spruce, is definite. Above it is the beech maximum, characteristic elsewhere and followed by a maximum of oak and hickory. Above this the cycle is again repeated, with a second beech maximum, followed once more by oak and hickory.

13. CANTON BOG.—This is very near the glacial limit. No fir is showing, but pine-oak maximum, followed in order by beech, oak, and hickory. The top of this deposit has been cultivated and doubtless also burned.

14. MOGADORE BOG.—The top is likewise destroyed, but the shift from fir to pine maximum is definite; likewise subsequent beech maximum, followed by oak and hickory.

15. FERN LAKE BOG.—Pine-oak maximum is very definite, with subsequent beech maximum, giving way to oak and hickory.

Discussion

Bog 8 lies in a region of coniferous forest. The remainder are in a region now characterized by deciduous forest—save no. 7, which is intermediate in position. Records of a formerly coniferous forest are present in all but nos. 5 and 6. In every instance this coniferous period was terminated by a strongly marked pine maximum. This is least marked in nos. 2 and 11. There is a concurrence of oak (often with hickory) and pine in nos. 3, 4, 7, 11, 12, 13, 15, and it is suggested in other profiles.

The pine maximum develops at the expense of fir in nos. 1, 4, 9, 10, 11, 12, 14, and 15; at the expense of spruce in nos. 1, 3, 4, 9, 10, 11, 12, and 15. The conclusion seems warranted that fir and spruce forests were replaced by pine. Spruce appears to bridge the transition from fir to pine. Generally speaking, this suggests a lowering of the degree of mesophytism. The usual order of succession is from pine to fir and spruce. This interpretation is strengthened by two circumstances: (a) the concurrence of pine and oak noted in the preceding paragraph and (b) the fact that the first pines to be represented are *Pinus banksiana* and *P. resinosa*. *P. strobus*, the most mesophytic, is most abundant toward the end of the pine maximum in the profiles studied. This is not shown in the diagrams.

Following the pine there is an increase of beech in nos. 2, 3, 4, 6, 7, 10, 11, 12, 13, 14, and 15. No. 1 shows the first appearance of beech at the pine maximum, but the record is truncated above that level. No. 5 begins above the pine maximum, and the only beech in the profile is at the base. No. 8 is not comparable, being in the coniferous region, while no. 9 has no beech and is truncated below the level at which beech probably first appeared. In every instance except these four,

the beech increases to a maximum, then decreases in favor of oak and hickory. The behavior of beech suggests an increase of mesophytism, followed by a lessening similar to that involved in the shift from fir and spruce to pine.

Profiles 2, 6, and 12 show a second maximum of beech above the first. But at the top, all profiles extending through deciduous time (nos. 2, 5, 6, 10, 11, 12, and 13) agree in showing a dominance of oak and hickory over beech. So far as beech indicates mesophytism, the present or recent past is a time of reduced mesophytism. Here again no. 8 is regarded as anomalous, being in a different environment.

COWLES (3) employed the term retrogression to designate a lowering of the level of mesophytism in a community. While chiefly concerned with this phenomenon in relation to physiographic change, he pointed out that it may be due to other factors and specifically mentions climatic change as one. The normal course of succession he recognized as being toward the greatest degree of mesophytism possible under existing conditions. Other terms applied to this phenomenon are regression, rejuvenation, and degeneration. The term employed by COWLES has the advantage of priority and clarity of definition. For this reason, although the writer is familiar with criticisms of the concept (2), he agrees with CAIN (1) in accepting the term.

The preceding discussion may therefore be summarized in the statement that twice during postglacial times there has occurred a retrogression in forest composition in the north central states. The first was a shift from fir through spruce to pine; the second a shift from beech to oak and hickory. Three of the seven profiles which extend to the present show a second appearance of beech followed by a third retrogression. Whether this third retrogression was due to local causes, or to general causes whose operation was buffered in four of the seven profiles which extend through deciduous time, is not clear.

In the observed course of plant succession, the movement is from less to more mesophytism. Relative stability is reached when the mesophytes present are able to reproduce themselves under conditions of their own domination. It follows that, if vegetation is replaced by that which is less mesophytic, a disturbance of the normal course of succession has taken place. Such a disturbance is implied in the two rather general instances of retrogression just described. Three obvious factors are involved in such a change. These are space, light, and moisture balance. Unless new areas are being opened up to vegetation in the vicinity, a pollen profile that shows a shifting vegetation must indicate that the new kind or kinds of vegetation are using the physical space occupied by the old.

In general, the scale of increasing mesophytism is paralleled by an increasing shade tolerance, or a decreasing light requirement. So far as this is true, the possi-

bility of retrogression would appear to be limited so long as the mature dominant mesophytes remain alive and in place, whether they are reproducing or not.

Both of these circumstances suggest strongly that retrogression from spruce and fir to pine, and later from beech to oak and hickory, must have been subsequent to the dying out, or killing out, of the more mesophytic forms.

It is not easy to postulate any biotic process or physiographic change so general in character as to account for such destruction and subsequent retrogression in the numerous localities involved. On the other hand, a less favorable water balance might produce just this result. Beech is notably sensitive to drought. Apparently the most reasonable explanation of the observed retrogression lies along this line.

The postglacial history of the Great Lakes is well known and has been marked by lowering of the drainage level. Conceivably this might have resulted in periods of rather general lowering of the water table. In view, however, of the variety of secondary drainage basins involved, the general low relief of the region, its generally adequate soil moisture before settlement, and particularly the continuous sedimentation within the basins studied, it may be doubted whether such recession of lake level would explain the retrogressions. On the other hand, these would be satisfactorily explained by periods of reduced humidity. This explanation is consistent with the presence of relict communities indicating one or more periods of relative dryness in postglacial times (4). And it agrees with the interpretation of Mud Lake (Ohio) profile (6). In this paper the writer classified the plants whose pollen was found on the basis of their supposed climatic significance. The conclusion was reached that there had been two periods of relative dryness, corresponding to those inferred from the present account of retrogressions.

In another paper (7) the writer compared the types of pollen profiles from various parts of eastern North America. With the exception of no. 8, those discussed in the present paper represent the "deciduous forest, central type" of the earlier report.

On the whole, the bogs of this region show little evidence of recent "climatic deterioration" in the sense of cooling and increased humidity following a postglacial warm, dry, or xerothermic period. The evidence of this is much clearer in the coniferous region bogs extending from Minnesota toward the Atlantic, where a maximum of oak is evident about mid-profile. This xerothermic oak maximum may correspond to the 18-foot level of our present no. 8 in northern Michigan.

The presence of "relict" species and communities, notably prairie, in the central deciduous area was responsible for the original hypothesis of a xerothermic period in North America. There has been no reason to assume that these western relicts were expanding aggressively at the time of European settlement. There is evidence to the contrary (4). So far as it has been studied, the general trend of plant succession in this region is normal—toward increased mesophytism. This

may have been accelerated by the cooling and increasing humidity evident farther north. Or it may merely represent ecological recovery following the shock of the second retrogression evident in the present data. In neither case does it appear to have produced any striking effect in the profiles here considered. More detailed study of profiles which are intact down to the present may shed light on this problem. Meanwhile, the two periods of forest retrogression described appear adequate to explain the presence in the central deciduous region of species and communities of more continental type than is now prevalent there.

Summary

1. Fifteen pollen profiles from bogs in the states of Illinois, Indiana, Michigan, and Ohio are figured, thirteen of which have not before been published. All but one are in the central deciduous region.
2. The forest sequence in these profiles shows two periods of retrogression. The earlier was a retrogression from fir and spruce to pine. This was followed by a relative increase of beech, ending in a second retrogression, when oak and hickory increased at the expense of beech. Three of the profiles show subsequent increase and retrogression of beech, but this does not appear to have been general.
3. The two general periods of retrogression are assumed to have been due to climatic causes, producing a less favorable water balance. This is considered adequate to explain the presence of xerothermic relicts in the central deciduous region.

The analysis of Fern Lake Bog by EVA GERSBACHER and of Camden Lake Bog by PRESTON SMITH were thesis problems. Much of the field and laboratory work was made possible by grants-in-aid from the Carnegie Institution of Washington.

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LITERATURE CITED

1. CAIN, S. A., The climax and its complexities. *Amer. Mid. Nat.* 21:146-181. 1939.
2. CLEMENTS, F. E., Plant succession. Carnegie Inst. Washington. 1916.
3. COWLES, H. C., The physiographic ecology of Chicago and vicinity: A study of the origin, development and classification of plant societies. *BOT. GAZ.* 31:73-108; 145-182. 1901.
4. GLEASON, H. A., The vegetational history of the middle west. *Ann. Assoc. Amer. Geog.* 12:39-85. 1923.
5. SEARS, P. B., A record of post-glacial climate in northern Ohio. *Ohio Jour. Sci.* 30:205-217. 1930.
6. ———, Pollen analysis of Mud Lake Bog in Ohio. *Ecology* 12:650-655. 1931.
7. ———, Types of North American pollen profiles. *Ecology* 16:488-499. 1935.

BEHAVIOR OF WOODY DESERT LEGUMES AT THE WILTING PERCENTAGE OF THE SOIL

R. F. DAUBENMIRE AND H. E. CHARTER

(WITH THREE FIGURES)

Introduction

According to many students of soil-plant relationships at the wilting percentage of the soil, the most drought-resistant of desert plants as well as the most drought-sensitive mesophytes should face a physiological crisis in their water economy at this point. In most mesophytes, such a crisis is easily recognized by a wilting of the leaf blades; but in many xerophytes, especially in sclerophyllous and succulent species, this usual manifestation of loss of turgor is lacking and others must be sought. Certain—if not all—of the woody desert legumes of the North American deserts are among this group of plants not exhibiting the usual symptoms of wilting.

ALWAY (1) planted woody desert legumes in metal containers, and after the plants were well established irrigation was withheld for a long period. He observed that *Prosopis velutina*, *Acacia greggii*, and *A. constricta* remain alive long after the soil moisture drops below the wilting percentage, and that during such protracted drought the tips of the shoots died, the lower leaves abscised, growth ceased, and the transpiration rate declined. In none of his experiments, however, did he attempt to correlate the relationship of these responses with the attainment of the wilting percentage of the soil and thus determine whether this point is of any more significance to these plants than are lower moisture percentages. It was with this question in mind that the experiments reported here were undertaken.

Material and methods

Palouse silt loam, into which was thoroughly mixed a small proportion of sand, was used throughout the experiments, and quart-capacity motor-oil tins were used for soil containers ("pots"). Before planting, the leguminous seeds were either notched with a file or treated with sulphuric acid, and with such treatments they germinated promptly. Three species of legumes were used: *Prosopis velutina* Woot., *Acacia farnesiana* Willd., and *Lysiloma thornberi* Britt. and Rose. All experiments were carried out in the greenhouse during midwinter or late spring, as will be discussed later.

The moisture content of the soil was brought up to 15 per cent, a point well

above the wilting percentage, at the beginning of the experiment.¹ This arbitrary percentage will subsequently be referred to as "favorable." Each pot was weighed daily, and twice the amount of water necessary to bring the moisture to 15 per cent was added. In this way the moisture content was kept fluctuating within narrow limits about the 15 per cent point, except in those pots where the plants were allowed to wilt.

A special type of irrigating apparatus was devised and used in all pots. Four test tubes with holes blown through the bottoms were tied into a bundle, and the four members of each bundle were staggered so that when the apparatus was placed in a vertical position in the soil, water added to each tube was delivered at a different level. One tube delivered water three-fourths of the distance down from the soil surface, a second delivered halfway down, the third one-fourth of the way down, and the fourth allowed water to trickle out just below the soil surface. Daily additions of water to each pot were divided equally among the four tubes. Preliminary trials with *Prosopis* indicated that this irrigation device is more effective in promoting rapid growth than adding all the water to the surface of the soil. No additional water was applied to compensate for the increasing weight of the plants as the experiment progressed.

Experimentation

The first series of experiments was directed toward the discovery of visible manifestations by the legumes of the attainment of that moisture level at which the leaf blades of mesophytes such as sunflower and wheat wilt permanently.²

From one to five leguminous plants were grown in each pot. After these had attained a height of approximately 20 cm., about five grains of wheat were also planted in each of the pots. When the wheat shoots were a few centimeters tall the surfaces of the pots were sealed with wax, and after they were about 15 cm. tall irrigation was suspended. Wheat, which shows wilting promptly and unmistakably, was used only as an indicator of that point in the gradual depletion of soil moisture referred to as the wilting percentage. It was assumed that the root systems of the leguminous and wheat plants growing in the same pot were so intimately associated that the moisture content of the soil would be depleted rather uniformly, and in consequence the water supply available to both species at any one time would be fairly equal. Observations of the degree of branching and the spread of root systems seemed to warrant such an assumption.

By observing the plants closely, it was possible with this technique to discover what definite reactions, if any, would be exhibited by the legumes when the wilting

¹ The field capacity (4, p. 370) of this soil was 19 per cent and the moisture equivalent 17.5 per cent.

² Permanent wilting is defined as a degree of wilting so complete that leaves will not regain their normal unwilted appearance after 24 hours in a moist chamber (2).

percentage of the soil was attained. Attention was directed particularly to changes: (a) in the appearance of the leaf blades, (b) in the normal day and night positions of the leaves, (c) in the rate of abscission, and (d) in the rate of terminal growth of the shoots.

As controls, an evenly matched series of pots containing legumes and wheat were planted and treated identically, except that the soil moisture was maintained in the vicinity of 15 per cent throughout the experiment rather than allowing the soils to desiccate. This series served as a basis of comparison to evaluate changes in appearance of the experimental plants. Also, these controls were necessary to demonstrate that the phenomena noted in the experimental plants could not be attributed to increasing age, increasing root competition, poorer soil aeration resulting from sealing the pots, or variation in greenhouse environment.

A second series of experiments considered the effect of the attainment of the wilting percentage upon the rate of transpiration in the legumes. Inasmuch as the weighing method was employed to measure transpiration, the technique of interplanting wheat could not be used here, because the water loss of the wheat plants could not be distinguished from that of the legumes rooted in the same soil. As a result of the first series of experiments, however, manifestations of a condition which corresponds to permanent wilting had been observed in the legumes themselves, and these could be used in correlating transpiration behavior with the attainment of the wilting percentage in pots containing only legumes.

In these transpiration experiments the soil surface was sealed with wax after the legumes had attained a height of approximately 20 cm. Each pot was weighed morning and night. After determining the normal diurnal and nocturnal rates of transpiration during a period when soil moisture was maintained at a favorable level, irrigation was suspended. The march of transpiration was then followed with reference to the development of symptoms of wilting such as were discovered in previous experiments.

An atmometer was operated simultaneously on the greenhouse bench throughout this second series of experiments.

Results

CHANGES IN APPEARANCE OF LEAF BLADES.—The leaf blades of the *Prosopis* plants, which were rooted in the same pot with wheat, exhibited no changes in appearance as the wheat leaves attained the state of permanent wilting and shortly thereafter died. Within a few days after the wilting percentage was attained in the *Acacia* pots, the leaves of this species had gradually assumed a dull, dead appearance and had become so dehydrated as to become brittle. The leaves of *Lysiloma* remained fresh in appearance long after the wilting percentage was attained. Just

before the pinnules of this plant were abscised, owing to extreme drought, they usually turned bright yellow.

CHANGES IN NORMAL DAY AND NIGHT MOVEMENTS OF LEAVES.—So long as *Prosopis* plants have access to an abundance of soil moisture, the pinnules regularly fold upward at night until paired pinnules are almost in contact with each other above the rachis of the pinna. As drought becomes acute this nyctitrophic response gradually becomes irregular; certain pairs fold while others remain expanded. Furthermore, this irregularity bears no correlation with day or night periods, and there was no consistency in behavior with different parts of the same plant or even with the same leaf.

Normally the pinnules of *Acacia* fold upward together at night, and at the same time the pinnae and petioles bend upward, giving the shoot a fastigiate appearance. When the wilting percentage is attained the leaves assume this nocturnal attitude and maintain it thereafter. The position of pinnae and pinnules in the daytime (fig. 1) is therefore an excellent indicator of whether or not there is growth water in the soil, that is, moisture in excess of the wilting percentages.

Under favorable moisture conditions the pinnules of *Lysiloma* also fold upward together at night and open widely at dawn. If insolation is not intense the leaves of this species remain expanded throughout the day; if insolation is intense, the nocturnal position is resumed a few hours after dawn, even though soil moisture conditions are favorable. Whether or not the wilting percentage has been attained can be determined by observing the plants after sunrise but before the sun is very high, since those plants which are rooted in soil which has reached the wilting percentage do not open their leaves during this period (fig. 2).

CHANGES IN GROWTH RATE.—When the soil moisture became so depleted that wheat wilted permanently, elongation of the shoots ceased abruptly in all three species. In *Prosopis* young leaves continued to expand somewhat after this point was reached, even though leaflets at the bottom of the shoot were abscising rapidly, owing to drought. The behavior of this species is different from that of maize plants as studied by DAVIS (3). The growth rate of maize decreases with each reduction of the soil moisture content below the capillary capacity and stops when the moisture content approaches a level of about 3 per cent above the wilting percentage.

CHANGES IN RATE OF ABSCISSION.—Daily abscission records of the control series (table 1) showed that there is a slow continual shedding of pinnules in vigorously growing plants of all three species of legumes. In such plants the rate of development of new leaves at the shoot apex far exceeds the rate of abscission at the lower extremity, so that the total leaf surface increases steadily. When the wilting percentage is attained, however, the production of new pinnules ceases and the shed-



FIGS. 1, 2.—Fig. 1, at left, wilted plants of *Acacia constricta* and wheat; at right, plants of same species in unwilted condition. Fig. 2, at right, wilted plants of *Lysiloma thornberi* and wheat; at left, unwilted plants of same species. Photograph made in early morning; a few hours later the pinnules on the unwilted plants of *Lysiloma* also folded together for the day.

ding of old ones increases sharply (table 1), so that the total blade surface is rapidly reduced. The method of recording abscission as the total number of leaflets dropped per day does not give as striking figures as would have been obtained had it been feasible to express abscission as a percentage of the total pinnules on the

TABLE 1

RECORD OF PINNULES DROPPED ON CONSECUTIVE DAYS BY EXPERIMENTAL AND CONTROL PLANTS. POINTS AT WHICH WHEAT WILTED PERMANENTLY INDICATED BY HORIZONTAL LINES. IRRIGATION IN THESE SERIES HAD BEEN SUSPENDED SEVERAL DAYS PREVIOUSLY. CONTROL SERIES IN EACH CASE CONSISTED OF PLANTS EVENLY MATCHED WITH EXPERIMENTAL SERIES FOR VIGOR AND SIZE

DAYS	TOTAL PINNULES DROPPED					
	PROSOPIS		ACACIA		LYSILOMA	
	SEVEN EXPERI- MENTAL PLANTS	SEVEN CONTROL PLANTS	FOURTEEN EXPERI- MENTAL PLANTS	FOURTEEN CONTROL PLANTS	FOUR EXPERI- MENTAL PLANTS	FOUR CONTROL PLANTS
1.....	12	1	2	0	5	5
2.....	4	1	1	6	6	4
3.....	9	0	2	6	5	7
4.....	6	1	0	28	9	15
5.....	4	0	19	2	12	7
6.....	9	4	35	27	16	34
7.....	10	3	8	17	0	1
8.....	43	3	78	34	11	8
9.....	44	4	176	11	6	11
10.....	24	7	188	30	31	118
11.....	25	4	386	18	466	10
12.....	43	9	229	11	80	9
13.....	39	6	221	8	82	11
14.....	60	7	178	32	56	15
15.....	61	3	88	2	46	4
16.....	65	2	418	41	49	17
17.....	17	5	818	33	56	18
18.....	67	13	154	31	52	10
19.....			1,179	15	39	6
20.....			1,352	10	289	8
21.....			1,182	5	215	8
22.....			1,259	8	103	15
23.....					79	42
24.....					29	6

plant, for with cessation of growth, abscission resulted in progressive elimination of pinnules. Even so, the sudden increase in number of pinnules lost per plant was maintained as long as the plants were observed after the wilting percentage had been attained. The irregularities which appear in the records of abscission rate were occasioned when an entire pinna or leaf dropped, carrying a number of pinnules with it.

EFFECT OF ATTAINMENT OF WILTING PERCENTAGE UPON TRANSPIRATION RATE.—Transpiration experiments with *Prosopis* were carried out in December and January, during which season the insolation at Moscow, Idaho, was very feeble and the photoperiod much shorter than that obtaining in the natural range of this species. In an attempt to compensate for such abnormal illumination, a 700-watt lamp, mounted under a reflector, was suspended 1 meter above the greenhouse bench upon which these plants were growing. This supplemental illumination was

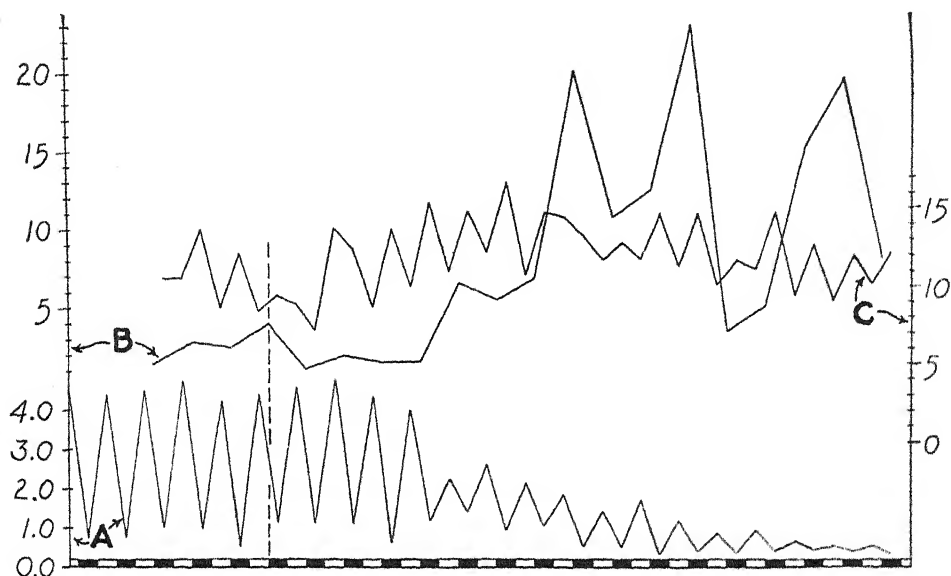


FIG. 3.—March of transpiration, abscission, and evaporation during experiments with *Prosopis*: A, transpiration in grams per plant; B, average number of leaflets lost per plant; C, atmometric evaporation in cubic centimeters. Alternation of night and day periods indicated at bottom of graph by alternating black and white lines, respectively. Point at which the last irrigation water was supplied is indicated by broken vertical line.

employed daily from 7:30 A.M. to 7:30 P.M., at which times the morning and evening weights of the pots were determined.

In addition to measurements of transpiration and evaporation, a record was kept of the rate of abscission, which in the preceding series of experiments proved a good indicator of the attainment of the wilting percentage in all three species of legumes.

Under conditions of favorable soil moisture and rather uniform illumination, the volume of water lost by *Prosopis* plants during the day was about five times as great as the nocturnal water loss (table 1; fig. 3). After observing this normal rhythm for 5 days, irrigation was suspended. The amount of moisture in the soil

at this time (approximately 15 per cent) was apparently sufficient to allow normal functioning of the plants for about 5 more days, at the end of which the rate of abscission suddenly increased and remained at a high level, and concurrently the rate of diurnal transpiration decreased sharply (fig. 3). The significance of the concomitance of these two observations is strengthened by the fact that there was no sharp decrease in the evaporative power of the air or changes in illumination which could account for the sudden change in transpiration rate. The results of this experiment seem to warrant the conclusion that the normal rate of transpiration in *Prosopis* continues during progressive desiccation until the wilting percentage is attained, at which time the water loss is abruptly reduced. These results are in accord with those of VEIHMEYER (5), who worked with young prune trees.

Under the range of evaporative intensities obtained in the greenhouse (fig. 3), transpiration appeared not to be governed by the evaporative power of the air as determined by an atmometer. In fact, transpiration was usually lowest when the evaporative rate was relatively high. Apparently transpiration was closely governed by the amount of illumination. On the other hand, in the absence of wind currents and with fairly uniform temperature, the evaporative rate depended chiefly upon relative humidity, which was usually highest during the day because the greenhouse was sprinkled in the morning. It would have been desirable in connection with these observations of transpiration to know the daily trend of stomatal movement in the legumes, but the pinnules were so small and delicate that none of the methods of studying stomatal apertures known to the writers proved successful.

Another notable feature of the transpiration behavior of *Prosopis* is that the nocturnal rate of water loss appears to be less dependent upon the existence of growth water than does the diurnal rate, although under prolonged drought even nocturnal transpiration is strongly reduced (fig. 3). This may be explained by the fact that at the wilting percentage the plant continues to absorb water, although at a very slow rate, and this quantity goes farther toward meeting the demands of the feeble nocturnal transpiration than of the more vigorous diurnal transpiration.

Experiments with *Acacia* and *Lysiloma* were carried out in May and June, when, except for an occasional cloudy day, insolation was of sufficient intensity and duration so that no supplemental illumination was considered desirable. The evaporation rate at this season varied directly with insolation and resultant temperature fluctuations within the greenhouse, the effect of the latter being so great that all other factors controlling evaporation were subordinated.

The transpiration rate of these two species likewise varied directly with the strength of insolation so long as the soil contained growth water. At this season transpiration was so vigorous that growth water was exhausted within about a day after progressive desiccation started. As in *Prosopis*, plants allowed to dry out ex-

hibited an abrupt decrease in transpiration, accompanied by a rise in the curve of abscission, indicating that the attainment of the wilting percentage is marked by sudden reduction in transpiration. These phenomena were concomitant with the anticipated behavior of pinnules, and in consequence it may be concluded that the results were essentially identical with all three species of legumes.

Summary

1. The wilting percentage of the soil has essentially the same significance in the water relations of *Prosopis velutina*, *Acacia farnesiana*, and *Lysiloma thornberi* as in wheat.

2. In contrast with wheat, the attainment of the wilting percentage in the legumes is not marked by a wilting of the leaf blades. However, the attainment of the wilting point brings about these changes in the legumes: (a) an essentially concomitant increase in the rate of abscission, (b) deviation from the normal diurnal positions of the pinnules, (c) cessation of shoot elongation, and (d) sharp decrease in the transpiration rate.

3. Apparently the growth and transpiration rates of the legumes continue at approximately normal levels as long as the soil contains any water in excess of the wilting percentage.

4. As long as the roots of the legumes have access to growth water, their transpiration rates appear to be closely related to the strength of illumination and to be unrelated to variations in the evaporative power of the air, so long as the latter is not likewise controlled principally by insolation.

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LITERATURE CITED

1. ALWAY, F. J., Studies in the relation of the non-available water of the soil to the hygroscopic coefficient. Nebraska Agr. Exp. Sta. Bull. 3. 1913.
2. BRIGGS, L. J., and SHANTZ, H. L., The wilting coefficient and its indirect determination. BOT. GAZ. 53:20-37. 1912.
3. DAVIS, C. H., Absorption of soil moisture by maize roots. BOT. GAZ. 101:791-805. 1940.
4. LOOMIS, W. E., and SHULL, C. A., Methods in plant physiology. New York. 1937.
5. VEIHMEYER, F. J., Some factors affecting the irrigation requirements of deciduous orchards. Hilgardia 2:125-284. 1927.

PECULIARITIES OF THE INFLORESCENCE IN THE EUPHORBIACEAE

LEON CROIZAT

(WITH FOURTEEN FIGURES)

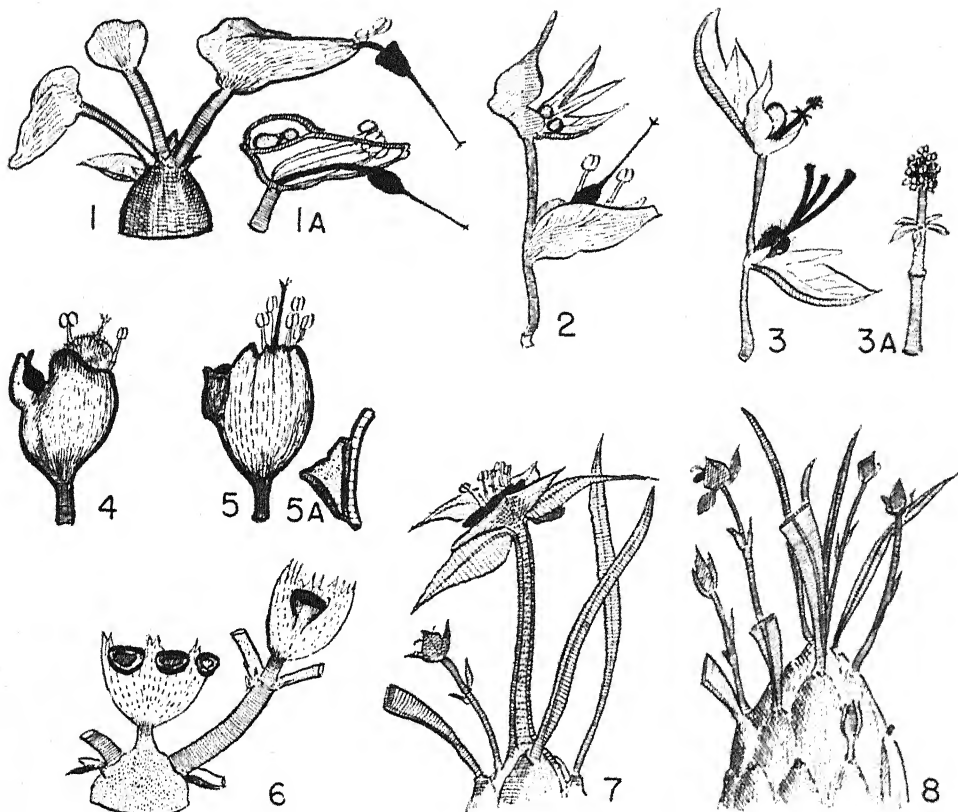
The cyathium of the Euphorbiaceae Euphorbieae is a structure unmatched by other inflorescences in the spermatophytes. It is the purpose of this paper to discuss certain of its aspects, concluding with a cursory review of the epicarp of *Ptychopyxis*, a genus which belongs to the same family if not to the same tribe. In a later paper attention will be given to the inflorescence of certain Celastraceae which suggests considerations of a general nature on the significance of flower-bearing axes.

I. Cyathium of *Pedilanthus*

The cyathium of *Pedilanthus* consists of two chambers (fig. 1A), one superior and smaller, containing a variable number of glands, the other inferior and larger, mostly tubular in its outline, holding the "ovary," that is, the ♀ flower and numerous stamens which in reality are monandrous ♂ flowers. This "flower" has been interpreted and described by students of the Euphorbieae (BOISSIER in DC. Prodr. 15(2):4. 1862; MILLSPAUGH in Field Mus. Publ. Bot. Ser. 2:353. 1913) in a manner which is hardly satisfactory. For instance, BOISSIER concludes that the upper lip is merely an external cucullate appendage becoming saccate at its base, including the glands and having no communication with the "tube" surrounding the sexual organs.

To elucidate the features of the cyathium of *Pedilanthus* which have proved embarrassing to BOISSIER and MILLSPAUGH, it is necessary only to tilt this peculiar inflorescence until the gynophore stands almost vertical. This done, it is necessary to suppose that the upper chamber is separated from the lower by a slight elongation of the pedicel. The diagram which figures the outcome of these assumptions (fig. 2) is strikingly reminiscent of the two-storied disposition characteristic for the inflorescence of another euphorbiaceous genus, *Dalechampia* (fig. 3). The floral structures of *Pedilanthus* and *Dalechampia* differ basically only as follows: (a) in *Pedilanthus* the upper chamber contains a variable number of glands but no active sexual organs; in *Dalechampia* the upper chamber contains active ♂ flowers in addition to more or less evolute glands; (b) in *Pedilanthus* the lower chamber contains one ♀ flower and numerous ♂ flowers; in *Dalechampia* the lower chamber is occupied by three ♀ flowers only. On this basis, if the upper chamber of the inflorescence of *Dalechampia* is deprived of its ♂ flowers and these

are placed around the central ♀ flower of the lower chamber (the lateral ones being suppressed), the cyathium of *Pedilanthus* is seen to appear. The changes hypothesized here to effect a passage from the inflorescence of one to that of the



FIGS. 1-8.—Fig. 1, inflorescence of *Pedilanthus tithymaloides*; A, section through cyathium showing upper chamber with two glands, lower with ♀ flower (ovary) and monandrous flowers (stamens). Fig. 2, diagram illustrating the presumed manner of formation of cyathium of *Pedilanthus*, the upper chamber being produced above the lower in the arrangement characteristic of the inflorescence of *Dalechampia*. Fig. 3, inflorescence of *Dalechampia*; A, ♂ flower. Fig. 4, cyathium of *Pedilanthus tehuacanus*. Fig. 5, same of *P. linearifolius*; A, section through gland. Fig. 6, diagram illustrating euphorbioid cyathium (with several glands) at apex of main stem of *Euphorbia geniculata*, followed above by pedilanthoid cyathium (with one gland) in fork of first dichotomy of umbellaster. Fig. 7, *E. clava*: apex of main stem bearing falsely apical cyathium set upon extension of podaria of main axis. Fig. 8, normal apex of main stem of *E. clava*.

other genus are consistent with the morphology and the sexual expression of the Euphorbiaceae, a family which frequently yields monoecious, dioecious, or polygamous inflorescences in the same genus and even in the same species.

The theory that the appendix of the cyathium of *Pedilanthus* is homologous with the upper bract that incloses the androecium of *Dalechampia*, and that the saccate chamber closed above by this appendix is actually a sterilized androecium but potentially a 1- or 2-sexual inflorescence, invites the objection that the hypothetical elongation of the pedicel above and beyond the lower chamber (fig. 2) is not to be taken for granted. Proof should be given that the stelar continuation of the pedicel reaches the upper chamber of the inflorescence. In view of this objection it must be admitted that the matter does not seem to have received as yet the attention of a specialized morphologist. However, a careful student of the vascular anatomy of the cyathium of *Euphorbia* (HABER in Ann. Bot. 39:704. 1925) has concluded that in this inflorescence the glands are "a pair of modified secondary branches of a lateral inflorescence"; that is, the glands are primarily caulinar bodies, unlike appendages of foliar origin. Since the glands of *Euphorbia* are often set upon the rim of the involucre in a manner hardly suggestive of stelar bodies, while those of *Pedilanthus* are often found at the bottom of the cavity of the upper chamber, it is not imprudent to assume, until proof to the contrary is given, that the supply of these glands is a branch off that of the pedicel of the inflorescence. Such an assumption has in its favor broad phylogenetic and morphological considerations, not less than the fact that the glands of *Euphorbia* and *Pedilanthus* are admittedly homologous.

Two modifications of the inflorescence of *Pedilanthus* are worthy of notice. In *P. tehuacanus* the tube is sharply truncated (fig. 4), the opening being almost vertical. The appendix is deeply cleft, and, so far as known, no glands are present in the upper chamber. The ovary is rounded as it usually is in *Euphorbia*, not elongate as in typical representatives of *Pedilanthus* (for instance, *P. tithymaloides*). In *P. linearifolius* the upper chamber is exceedingly reduced, appearing as a glandular saccate fold on the side of the vertical, baglike involucre (fig. 5). The tendency inherent in the cyathium of *P. tehuacanus* to depart from zygomorphism with the concomitant reduction of the upper chamber suggests that a further evolution in this type of cyathium may yield a vertical, exappendiculate and eglandular perianth, such as exists in the ♂ cyathium of *E. plagiantha*. This suggests that a shift in the position of the floral axes may have far-reaching consequences upon the nature of specialized reduced inflorescences.

The cyathium of *P. linearifolius*, a species which some treat as *Cubanthus linearifolius*, does not differ in its essential characters from the cyathium of *Euphorbia* (*Poinsettia*) *pulcherrima*. These two species agree closely in floral and vegetative characters and to all appearances are consanguineous. It is possible that the solitary nectary of the cyathium of *E. pulcherrima* is not absolutely homologous with the saccate lateral appendage of that of *P. linearifolius*, and that the former is a much more reduced and specialized structure than the latter. Both

these "glands" have the function of hydátodes, however, which sets them physiologically on a par; it should not be surprising that they differ in morphological details, because relevant differences separate the various kinds of nectaries and glands prevalent in *Euphorbia*. The glands of *E. esula*, *E. jacquiniiflora*, and *E. tridentata* are most unlike, but differences of the kind are correctly interpreted as indicating little that has absolute value in a phylogenetic study. To judge from a sum of characters, *P. linearifolius* and *E. pulcherrima* are the links connecting *Pedilanthus* and more distantly *Dalechampia* with the aggregate of species commonly treated by taxonomists as *Euphorbia*. The phylogenetic significance of the so-called section *Poinsettia* of *Euphorbia* is certainly greater than most taxonomists appear to believe, as it is through this section that the prevailingly actinomorphic cyathium of *Euphorbia* can be connected in phylogeny with the zygomorphic inflorescence of *Pedilanthus*, and—more remotely—with the ancestral inflorescence common to all the Euphorbieae. In this connection it is interesting that the species of section *Poinsettia* are closely related with African and Australian forms in the vicinity of *E. crotonoides* and *E. eremophila*, forming an aggregate (in the broad sense) that enjoys practically a pandemic range.

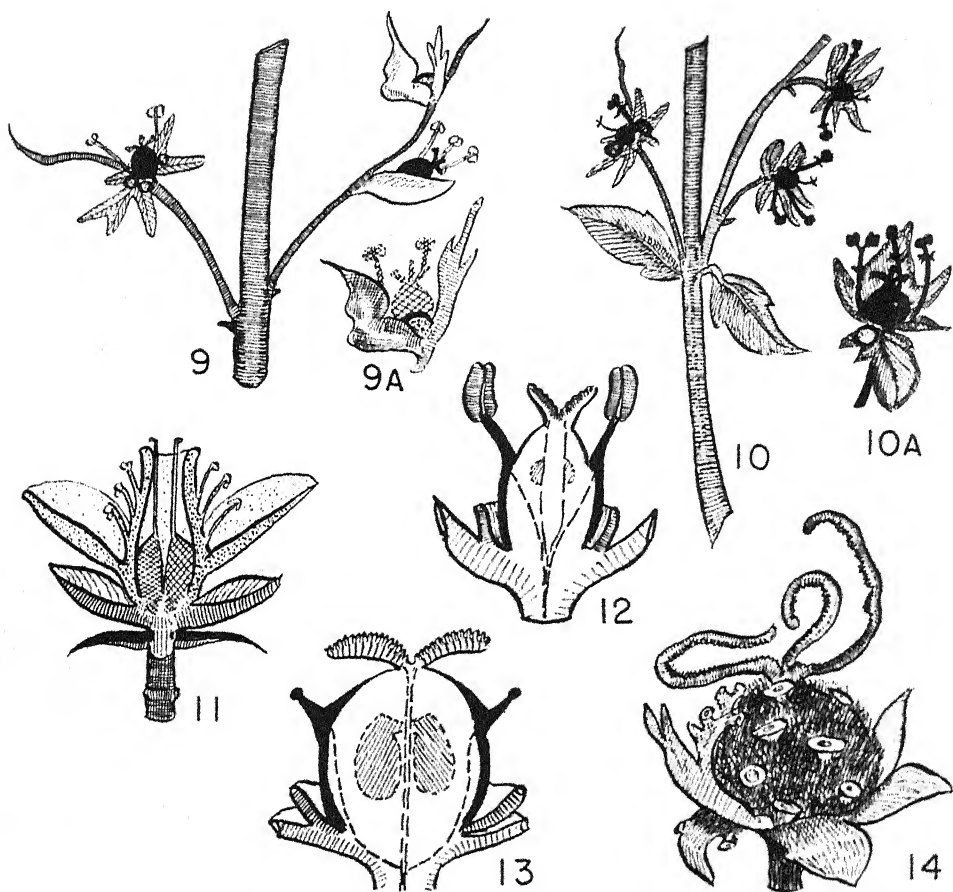
In view of the position occupied by the species of section *Poinsettia* among the Euphorbieae, it is not surprising to find in these species dimorphic cyathia of considerable interest. In *E. geniculata*, for instance, the primary main stem often ends with a cyathium that may have eight to ten glands; next above, at the first dichotomy of the umbellaster, cyathia occur which are typical of the section and bear only one gland, being the same as those of *E. pulcherrima*. This peculiar arrangement (fig. 6) suggests that in the species of this section a progressive specialization takes place at different levels on the floriferous axes, the same species having first a euphorbioid cyathium (with more than four glands), then poinsettoid cyathia (with only one gland). Such a specialization, as has been noticed, also occurs in *Pedilanthus* (fig. 2), in which the lower chamber is sexually complete and active, the upper sterile, and occupied only by relic bodies of the nature of glands. Moreover, the first cyathium to appear in species of *Euphorbia* outside of section *Poinsettia* is often unlike the cyathia that follow it. This first cyathium may be unisexual, have nearly free glands and lobes, and especially eight to ten glands and lobes instead of the normal number of four to five. In one species, *E. capitulata*, no umbellasters form, each fertile shoot being terminated by a single cyathium. This cyathium has a status and position closely corresponding to those of the first cyathium of other species of *Euphorbia*. It arises at the origin of the umbellaster, and, like it, has usually eight to ten lobes and glands, a peculiarity which SCHMIDT has used to erect a monotype, *Diplocyathium*. Apparently a cyathium with four to five lobes and glands is not necessarily characteristic of the species of *Euphorbia*, and the cyathia of this genus and of the Euphorbieae in general tend toward dimorphism.

In her study of the cyathium of *Euphorbia*, HABER has concluded (Ann. Bot. 39:694. 1925) that the articulation of the monandrous pseudo-stamen of this peculiar inflorescence is "the receptacle upon which the flower is situated, and in function is similar to that of other receptacles." Since the receptacle may be understood as a region in which earlier growth comes to a stop and new growth begins, it is logical to suppose that a flower that is apparently terminal past an articulation is not necessarily apical. This flower may arise, in fact, from a meristem that is lateral upon the receptacle, occupying an apparent terminal position on the axis on account of the failure of the true apical meristem to develop a flower. An articulation, consequently, may be analogous if not homologous with the scar that marks the insertion of a scion upon the stock to which it has been grafted, and it is not surprising that it should be anatomically complex (LECOMTE in Mus. Nat. Hist. Paris, Nouv. Arch., 5 sér. 2:121-242. 1910). In theory at least, an articulation does not require that the axis come to an abrupt end, establishing a sharp horizontal receptacle. It may form when a lateral meristem acquires preponderant growth, as when, for instance, a lateral bud of *Tilia* or *Ulmus* becomes "terminal" on the shoot on account of the dying-out of the apex of the branchlet upon which this bud is borne. The formation of an articulation may consequently be regarded as the result of a theoretical reversal in the normal ratio of development between the bud gap and the stele, the stele being ultimately disposed of by too continuous a proliferation of lateral buds. In the physiological sense, this happens particularly when dormant structures gain the upper hand against active ones at the end of a length or time of growth. There does not seem to be a sharp line to separate such true articulation, as that of the monandrous flower of *Euphorbia*, described by HABER, from the abscission layer and scar which end a branchlet of *Ulmus* or *Tilia* next above a falsely terminal bud.

The cyathium of *Euphorbia* is apparently terminal, and its pedicel does not exhibit a manifest articulation. It is very likely, however, that this cyathium is not truly apical. *Euphorbia clava*, a succulent which is often cultivated as an ornamental, has main axes which normally do not bear apical cyathia (fig. 8), these being carried upon lateral specialized floriferous axes. In some cases, however, the main stems taper to a peduncle-like end (fig. 7), crowned by a cyathium. Of course this cyathium is not truly apical, because it rests upon a structure of spirally ascending podaria (=decurrent succulent petioles) and is in reality an intercalary inflorescence (PARKIN in Jour. Linn. Soc. Bot. 42:556-558. 1914). What seldom happens in *E. clava* normally takes place in *E. caput-medusae*, in which all cyathia are borne upon peduncles of spirally ascending podaria. In a species in the vicinity of *E. clava* (probably *E. loricata*) I have found by dissection that the bottom of the involucre actually slants upon the supporting axis, showing that the cyathium is not really apical. Thus evidence is forthcoming that the

cyathia of the Euphorbieae are not necessarily and truly apical, despite their appearing in a terminal position upon the axes.

Once it is realized that these peculiar inflorescences are essentially intercalary, that is, that they become falsely terminal by the extinction of the axis which bears



FIGS. 9-14.—Fig. 9, diagram illustrating components of cyathium of *Euphorbia geniculata*; A, outline of gland occupying place of sexual organs in upper chamber (=upper chamber of cyathium of *Pedilanthus*; see fig. 2). Fig. 10, illustrating presumed ancestral inflorescence of Euphorbiaceae Euphorbieae; A, detail of glomerule. Fig. 11, *Pavonia hastata*: diagram of flower in longisection. Fig. 12, *Cleistanthus decurrens*: diagram of ♂ flower with pistillode. Fig. 13, showing possible evolution of the ♂ flower of *C. decurrens* by return to functionality of ♀ organs and persistency upon the ovary of sterilized stamens (= staminodes or processes of glandular nature). Fig. 14, flower of *Ptychopyxis thwaitesii* (*Podadenia thwaitesii*) showing presence of actual glands on epicarp.

them in a lateral position, it is not difficult to reconstruct the hypothetical ancestral inflorescence which gave origin to any and all forms of cyathium, that of *Pedilanthus* together with that of *Poinsettia* and *Euphorbia*. The dimorphic

cyathia of *E. geniculata* (fig. 6), for instance, may be homologized to three lateral inflorescences (fig. 9). Of these three, one yields an euphorbioid cyathium by the simple process of fusion of glands and bracts (lobes), four to ten glands becoming adnate and alternate with as many bracts. On the contrary, the pedilanthoid cyathium with one gland is formed by the specialization and subsequent fusion of two inflorescences in a manner characteristic of *Pedilanthus* (figs. 1, 2). In brief, the dimorphism observed in the cyathium of the Euphorbieae in general is the result of the specialization of the inflorescences ancestral to the cyathium, these inflorescences becoming cyathia either as single units or by fusion of two units, it being probable that the euphorbioid cyathium with many glands arises from one inflorescence and the pedilanthoid cyathium with no apparent glands or one gland from the fusion of two inflorescences. The fundamental units in all these inflorescences are the following: (a) a central ♀ flower with a much reduced calyculus; (b) a variable number of ♂ flowers, with or without a calyculus, bearing one to several stamens; (c) glands representing abortive ♂ flowers; (d) a variable number of appendages. On this basis the ancestral inflorescence of the Euphorbieae and Dalechampieae, and possibly of the Perea, might have resembled the inflorescence shown in figure 10. Such an inflorescence is aptly described (BAILLON in *Adansonia* 6:354. 1866) as being composed of "Cymes pluripares à fleur femelle centrale," and some of its earliest stages may not have been altogether different from the floral arrangement of *Longelia buxoides* as defined by BAILLON. All these inflorescences relate back to the capitula of the Tiliaceae and to the cymes of the Malvaceae, modified by sexual specialization and by extensive fusion among partial inflorescences and single flowers. Thus the phylogeny of the inflorescences of the Euphorbieae confirms what is known on the strength of other evidence, that the Euphorbiaceae and the Sterculiaceae are closely related, both families arising from the malvoid ancestral group as a case of sexual specialization.

II. Epicarp of Ptychopyxis

The corolla of malvaceous flowers is described in most textbooks as dialipetalous. This is erroneous, as the corolla in these flowers is intimately connate with the staminal column, the petals and the androecium falling together, calyptra-wise, after anthesis. Such flowers in longitudinal section have an outline similar to that shown in figure 11.

Pavonia hastata, a species of the Malvaceae, is known to turn "cleistogamous" at certain times of the year (GOEBEL in *Organ. Pflanzen*. 3 ed. 3:1971 [fig. 2035]. 1933, CROIZAT in *Darwiniana* 5:419. 1941). This "cleistogamy" actually consists of a reduction of the corolla and staminal column which, instead of evolving in full blossom, fail to develop and remain inclosed within the barely opened or even unopened calyx. That this is hardly a case of true cleistogamy need not be emphasized. What actually takes place in *Pavonia* is this: in summer the corolla and

the staminal column develop in full, but for some reason or other the gynoecium remains sterile and no seeds are produced; in winter, on the contrary, the corolla and the staminal column become abortive but the pollen and the ovules are active, so that normal seeds capable of germinating are produced. The background of this differential growth would seem to be metabolic because it is connected with changes in average temperature. The tentative conclusion may be advanced that *P. hastata*—and with it other Malvaceae such as *Sida* spp.—tends toward a mode of sexual expression which involves a departure from the usual bisexual arrangement of the family. This tendency calls into play apetalous and unisexuality.

A malvoid entity which becomes completely apetalous and unisexual is turned, *ipso facto*, into a sterculiaceae and euphorbiaceae plant because the Sterculiaceae in part and the Euphorbiaceae as a whole differ essentially from the Malvaceae in being apetalous and unisexual. No sharp morphological line can be drawn to separate the Malvaceae, Tiliaceae, Sterculiaceae, and Euphorbiaceae, for they differ in tendencies more than in characters. Thus *Pavonia* and *Sida* illustrate the beginning of a type of floral evolution which eventually ends by establishing the cyathium of *Euphorbia*. The first step in this evolution is probably taken when the corolla and the staminal column become intimately connate, for it is then that a type of flower which is found in the Theaceae and, farther still, in the Magnoliaceae, becomes actually malvoid. As a matter of fact, the entire evolution may be tentatively outlined as follows: (a) the syncarpic, conelike gynoecium becomes reduced, only the basal row of carpels surviving either dialicarpically or syncarpically; (b) the stamens fuse together and with the corolla; (c) either the corolla or the staminal column evolves, ultimately leading to unisexuality; (d) unisexuality being attained, the flower undergoes further reduction in detail. All these steps merely postulate concentration and specialization of the various floral organs, that is, an essential progression in reduction. It is not surprising that in the families in which unisexuality and reduction prevail, such as the Sterculiaceae and the Euphorbiaceae, nectaries are frequently found together with pistillodes, these organs representing surviving but degenerate parts of the androecium or of the gynoecium.

In *Pavonia hastata* the gynoecium may become sterile, either anatomically or physiologically, this being a point upon which no information seems as yet available, and the androecium (corolla and staminal column) may become reduced, simulating cleistogamy and actually presenting a form of apetalous. In numerous Euphorbiaceae the ♀ organs are abortive and reduced to pistillodes or "glands," while the ♂ organs are fully developed, the phylogenetic link between the Malvaceae and the Euphorbiaceae being furnished by the Sterculiaceae. An especially revealing example of flower in which a pistillode is surrounded by stamens is that of *Cleistanthus decurrens* (fig. 12), a euphorbiaceous plant.

In the light of the tendencies prevalent in the malvoid-euphorbioid phylum it is permissible to suppose that the pistillode of *C. decurrens* might again become sexually active and anatomically perfect in connection with the process of ovule- and seed-bearing, the stamens being turned into staminodes, that is, into sterilized stamens or glandular bodies. As the result of this supposition a structure is visualized of the kind shown in figure 13, in which the staminode or its equivalent, a gland, persists upon the ovary and the fruit as a process or an appendage, the androecium (staminal column and corolla) becoming part of the epicarp.

The hypothesis that the processes upon the capsule of certain Euphorbiaceae are actually relics from an abortive androecium may not be true in every case, but it certainly explains manifestations which are otherwise difficult to account for. In certain species of *Codiaeum*, for instance, the epicarp is beset with glands that do not differ in the slightest from floral nectaries. Such nectaries are numerous upon the ovary and fruit of *Ptychopyxis thwaitesii* (fig. 14) and are in no wise distinct from the staminodes and glands normally situated next to the calyx. It is well known, moreover, that in *Ricinus* the prickles upon the capsule have a vascularization of their own which is not suggestive of epidermal processes as such. In conclusion: some evidence is forthcoming that in the malvoid flower and its derivative structures, the carpels as well as the stamens may become intimately connate and adnate with the corolla, which is fully in harmony with the tendency toward reduction and unisexuality prevailing in the malvoid and euphorbioid phylum. Since it is admitted—on the mere strength of visual evidence—that the abortive gynoeceum of the Euphorbiaceae persists as a pistillode (*Cleistanthus*) or as discrete glands (*Tetracoccus*, *Securinega*) in the ♂ flower, it should not be surprising that the androecium may persist as glands or other processes, free from the epicarp (*Croton*) or intimately connate with it (*Ptychopyxis*) in the ♀ flower.

Summary

1. The cyathia of *Euphorbia* and *Pedilanthus* represent different degrees of adaptation and fusion in a single type of compound lateral inflorescence. Such an inflorescence was probably intercalary in its origin.
2. A tendency toward the reduction of floral parts involving apetalry and asexuality appears in the Malvaceae and becomes dominant in the Sterculiaceae and the Euphorbiaceae.
3. The glands and processes on the epicarp of *Codiaeum*, *Ptychopyxis*, and *Ricinus* are apparently relics from the androecium that became intimately fused with the carpels. Glands of the same nature probably appear also in other families (Sabiaceae).

EFFECTS OF PHOTOPERIOD ON SEX EXPRESSION IN AMBROSIA TRIFIDA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 542

LOUIS K. MANN

(WITH FIVE FIGURES)

Introduction

It is known that photoperiod may exert marked influence upon the sex expression of certain monoecious and dioecious plants (5). In *Zea* and *Cannabis*, for example, shortening the photoperiod to less than that to which the plants are usually exposed at the time of flowering causes a "reversal" of the staminate (*Zea*) (8) or both staminate and pistillate (*Cannabis*) flowers (6, 7). In experiments carried out to determine the critical photoperiod of giant ragweed, *Ambrosia trifida* L., a short-day plant, it was noticed that among plants exposed to relatively short photoperiods (less than 9 hours) there was a reduction in the number of staminate involucre relative to the number of pistillate involucre, and in some cases racemes which were usually staminate contained some pistillate involucre.

The following experiments were designed to determine to what extent sex expression in giant ragweed could be modified by photoperiodic treatment, and, in this respect, to compare the effects of varying both the length of photoperiod and the number of photoinductive cycles to which the plants were exposed.

Material and methods

Experiments were carried out in the greenhouses at Chicago from June until October, 1941. Seeds were collected locally in the autumn of 1939 and kept in the refrigerator to break dormancy (2). The seeds were planted in flats on June 16, and the seedlings were later transplanted to 4-inch pots. The pots were placed on benches under natural daylight with the photoperiod extended until 10:00 P.M. On July 18 the photoperiod was further extended until midnight by Mazda light (200-watt bulbs in porcelain reflectors, kept 1-2 feet above the tips of the plants). Plants were selected for uniformity and treatments were started July 18.

In addition to the long-day bench, photoperiods of 15, 12, and 6 hours were used. The 12- and 15-hour photoperiods were obtained by covering frames on the greenhouse benches with a layer of black sateen cloth at 6:00 P.M. and 9:00 P.M., respectively, and removing the cloths at 6:00 A.M. the next day. Plants on the 6-hour photoperiod were placed in a darkroom between 5:00 and 6:00 P.M. and were returned to the greenhouse between 11:00 and 12:00 A.M. the following day. This

procedure for the longest dark period avoided the high temperatures which develop under black cloth during daylight.

The plants were treated in units consisting of approximately 100 plants; in all, ten of these experimental units (1000 plants) were used. The units were given the following treatments. One unit remained on the long-day bench until the end of



FIG. 1.—A, racemes of staminate involucres terminating main axis and lateral branches; pistillate involucres in leafy bracts below. B, suppression of long laterals common in greenhouse plants.

the experiment as a control. The remaining nine units were divided into three groups of three units each. The first group of three units was exposed to 15-hour photoperiods, one unit receiving two cycles, one receiving ten cycles, and the third receiving twenty cycles of this photoperiod before being returned to the long-day bench, where they all remained until the end of the experiment. In the second group the units were exposed to two, ten, and twenty cycles of 12-hour photoperiod before being returned to the long-day bench. The third group was similarly exposed to two, ten, and twenty cycles of 6-hour photoperiod and then returned to the long-day bench.

In general there was marked variation, even among plants within a single uniform treatment. In extreme cases, plants bearing fruit and other plants just beginning to differentiate flower primordia were found within a single unit. This variation necessitated the use of the large numbers of plants in each unit.

Results

In comparing ragweed plants which have received various specific photoperiodic treatments with field plants, it must be recognized that environmental factors

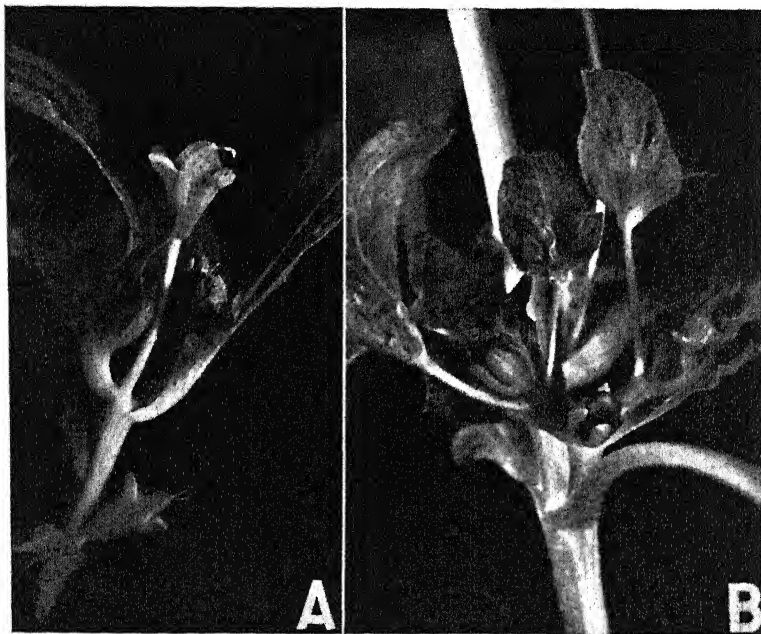


FIG. 2.—*A*, elongation of branch bearing pistillate involucres. *B*, cymose arrangement of pistillate involucres.

other than photoperiod alter the appearance of the plant when it is grown in the greenhouse. Since, in the ragweed, staminate and pistillate flowers are located on different parts of the plant, changes in vigor, height, branching, etc., may affect the relative proportion of pistillate and staminate flowers. Plants in the field have a dominant central axis with well-developed lateral branches which branch again several times. The terminal portion of the primary axis and those of most lateral branches are racemes of involucres of staminate flowers. The sessile or subsessile pistillate involucres are in small cymose clusters, each involucre being subtended by a leafy bract. For the most part these short cymose branches occur on secondary branches, or occasionally on the main axis as supernumerary branches in a

leaf axil immediately below the main branch in that axil. The racemes of staminate involucres and the leafy bracts containing the pistillate involucres are shown in figure 1A.

In general, plants grown in the greenhouse vary from field plants in the absence of most or all of the long secondary branches on the main axis (fig. 1B). The sec-

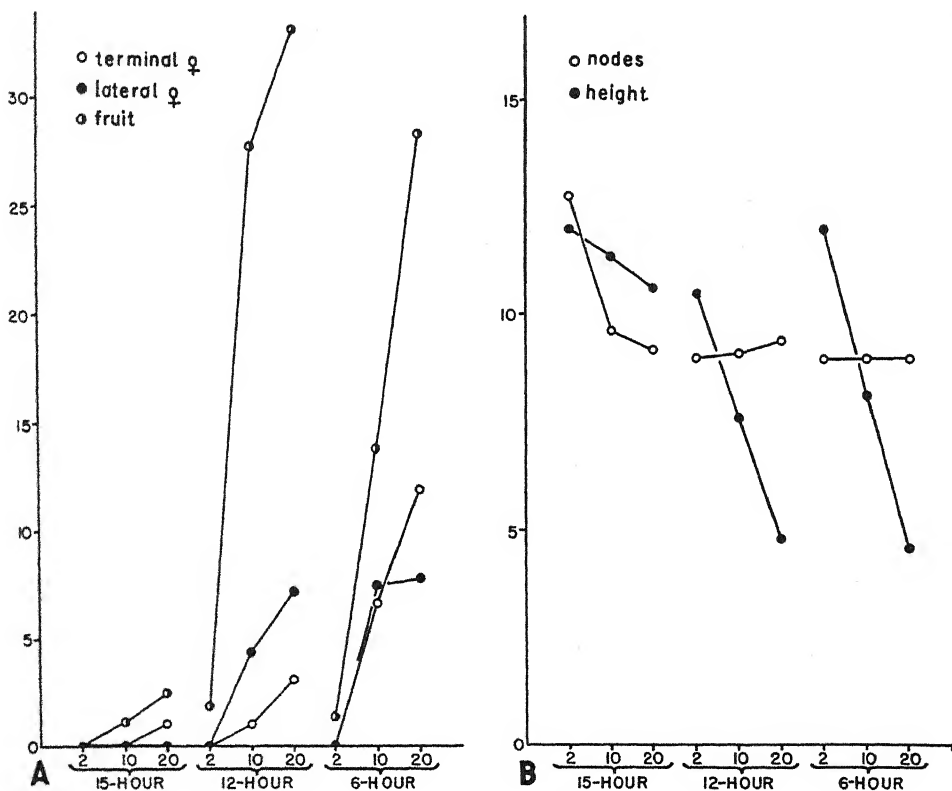


FIG. 3.—A, number of mature fruits per plant; number of terminal and lateral staminate racemes per 100 plants in which some or all staminate involucres were replaced by pistillate involucres. B, number of nodes and average height in decimeters of plants at maturity.

ondary branches, which are usually rather long, may fail to elongate, the terminal racemes being present but aborted. In other cases the terminal portion of the lateral branch may be replaced altogether by a typical cymose pistillate branch (fig. 2B). Occasionally such a branch (fig. 2B) may elongate (fig. 2A).

In so far as the primary interest in the plants was in the flowering and fruiting structures, most of the data were taken when the plants were mature (fruits ripe and leaves beginning to brown). In those groups which received only two photo-inductive cycles, regardless of their length, the maturation was so slow that the

plants were harvested before maturity. Thus the data from these units, with respect to number of mature fruits and height, are not wholly comparable with the other units. The control unit was macroscopically vegetative at the time of harvest, but on dissection most plants were found to be initiating flower primordia. This was probably the result of an irregularity in the electric-power supply which operated the lights. Previous work on ragweed seems to indicate that it remains vegetative under photoperiods of less than 9 hours.

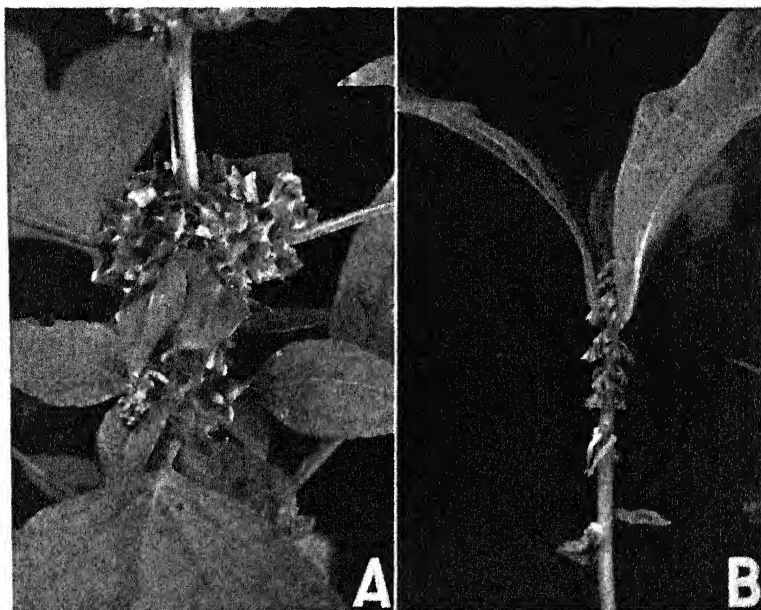


FIG. 4.—*A*, large clusters of pistillate involucres in leaf axil on main axis of plant. *B*, reversion of terminal raceme to vegetative condition.

Rate of maturity and intensity of photoperiodic response are probably best indicated by the height of the plants (fig. 3*B*). Uniformity in number of nodes indicates simultaneous induction of most units, the variation in height being due primarily to the length of the internodes. On the basis of rate of maturity, the unit given twenty 12-hour photoinductive cycles was induced most strongly, this group—on the average—maturing several days before any of the other units.

With respect to yield of fruit (fig. 3*A*), the unit receiving twenty 12-hour photoinductive cycles is again greater than the others. In this group the short cymose branches at the nodes of the main axis were well developed. A cluster of these branches with its many fruits is shown in figure 4*A*. In the units which received

only two photoinductive cycles, these short pistillate branches were poorly developed, usually not more than six fruits at a node.

Although the yield of fruit may be an indication of the balance between pistillate and staminate flowers, it cannot be taken directly as an indication of differentiation of staminate structures instead of pistillate. Although the position of pistil-

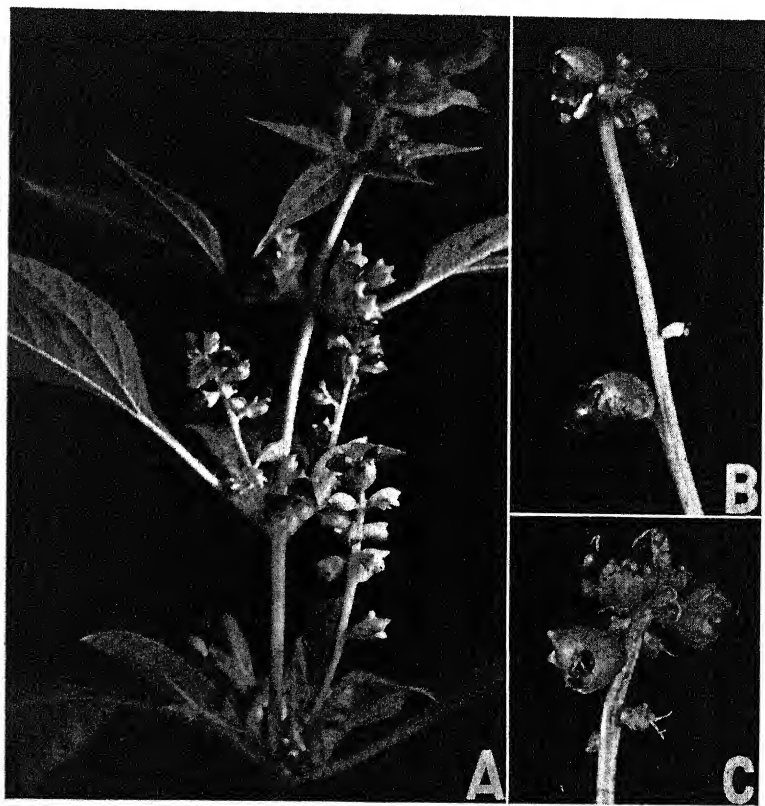


FIG. 5.—*A, B*, lateral racemes in which staminate involucres have been replaced by pistillate involucres. *C*, tip of terminal raceme with replacement of staminate involucres by pistillate involucres.

late involucres varies, even in field plants, the staminate involucres are confined to terminal racemes, either of the main axis or of the lateral branches. In plants which received short photoperiods, the staminate involucres in these racemes failed to appear, pistillate flowers developing in the position usually occupied by staminate. The development of pistillate involucres on the lateral racemes is shown in figure 5*A, B*, while a portion of a terminal raceme with differentiation of pistillate flowers is shown in figure 5*C*. Figure 3*A* shows the increasing incidence of pistillate involucres in racemes with increasing numbers of photoinductive cy-

cles and decreasing lengths of photoperiod. In several plants both terminal and lateral racemes developed pistillate involucre, resulting in completely pistillate plants.

In addition, various gradations between staminate and pistillate structures were found. Reversions to vegetative conditions (fig. 4B) were also common under short photoperiods. Variations of this type in other plants have been rather thoroughly described (1). The presence of simple leaves rather than the usual lobed type is not uncommon in plants in the field (3). Percentage of leaves which were simple varied from 18 to 31 under different treatments, the higher percentages being found in the lots which received smaller numbers of photoinductive cycles.

In *Ambrosia elatior*, variations in the inflorescence are found in populations growing in the field. These are similar to those brought about in *A. trifida* by very short photoperiods. JONES (4, 5) has demonstrated that the variations in *A. elatior* are associated with genetic forms. He has found that long photoperiod may modify expression in some strains of *A. elatior*, but that the usual monoecious form which breeds true was stable with respect to environmental changes. JONES found that *A. elatior*, of any genetic form, when subjected to short photoperiod was dwarfed and might fail to produce staminate involucre, but that "such pistillate plants, in a culture which should be monoecious, cannot be regarded as 'sex reversals,' since pistillate flowers have not replaced staminate flowers." In *A. trifida*, on the other hand, length of photoperiod and number of photoinductive cycles have a pronounced effect on sex expression. Lacking experimental information, the uniformity in field plants would suggest that genetic factors are of less importance in *A. trifida* than in *A. elatior*.

Summary

1. *Ambrosia trifida*, a short-day plant, after starting growth on long day, was subjected to two, ten, and twenty photoinductive cycles at each of three photoperiods—15, 12, and 6 hours. Following this treatment, the plants were returned to a long-day bench to complete growth.

2. Rate of maturity was most rapid and yield of fruit was greatest in the plants exposed to twenty photoinductive cycles at the 12-hour photoperiod.

3. Sex expression as measured by development of pistillate flowers in the position usually occupied by staminate increased with increasing numbers of photoinductive cycles and decreasing lengths of photoperiod, being greatest in the plants exposed to twenty photoinductive cycles at the 6-hour photoperiod.

LITERATURE CITED

1. BIDDULPH, O., Histological variations in cosmos in relation to photoperiodism. BOT. GAZ. 97:139-155. 1935.
2. DAVIS, W. E., Primary dormancy, after-ripening, and the development of secondary dormancy in embryos of *Ambrosia trifida*. Amer. Jour. Bot. 17:58-76. 1930.
3. DEAM, C. C., Flora of Indiana. Department of Conservation, Division of Forestry, Indianapolis. 1940.
4. JONES, K. L., Studies on *Ambrosia*. I. The inheritance of floral types in the ragweed, *Ambrosia elatior* L. Amer. Mid. Nat. 17:673-699. 1936.
5. ———, Studies on *Ambrosia*. II. Effect of certain environmental factors on floral development of *Ambrosia elatior*. BOT. GAZ. 98:296-306. 1936.
6. LOEWING, W. F., Physiological aspects of sex in angiosperms. Bot. Rev. 4:581-625. 1938.
7. SCHAFFNER, J. H., The influence of relative length of daylight on the reversal of sex in hemp. Ecology 4:323-344. 1923.
8. ———, Sex reversal and the experimental production of neutral tassels in *Zea mays*. BOT. GAZ. 90:279-298. 1930.

EFFECT OF CHEMICAL TREATMENTS IN PROLONGING DORMANCY OF TUNG BUDS

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AND FELIX S. LAGASSE⁴

(WITH TWO FIGURES)

Introduction

Several investigators have reported experiments in which the growth of buds was inhibited by treatment with various organic compounds. Among the first to propose a theory to account for the dominance of terminal buds in plants was LOEB (6). He suggested that there might be a substance moving downward from growing apical buds which inhibits growth of the lower buds. This theory is supported by the studies of THIMANN and SKOOG (9). GUTHRIE (2) noted that the potassium salt of alpha naphthalene acetic acid inhibited growth of buds in "non-dormant" potato tubers. Later, GUTHRIE (3) also observed that the vapors of the methyl ester of alpha naphthalene acetic acid produced the same effect. MITCHELL and STEWART (7), studying the growth responses produced by plant hormones, found that alpha naphthalene acetamide and alpha naphthalene acetic acid inhibited development of the terminal buds and expansion of primary leaves when applied to green plants in strong concentrations in the form of emulsion sprays. About the same time LINDNER (5) found that high concentrations of indoleacetic acid and alpha naphthalene acetic acid in lanolin (smeared on cut surfaces) increased the quantity of roots of horseradish but decreased the number of shoots in segments of the root. Recently WINKELPLECK (10) reported delaying the blossoming of excised branches of stone fruits for several days with various derivatives of alpha naphthalene acetic acid.

Whether the work of GARDNER, MARTH, and BATJER (1) and others on the inhibition of preharvest drop of apples by treatment with such compounds as alpha naphthalene acetic acid is a growth inhibition phenomenon is not clear at this time.

One of the most important problems confronting the tung industry today is the injury to the flowers by late spring frosts. During 2 out of the last 5 years, late spring frosts have very seriously reduced the production of tung oil in America.

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It is safe to say that if this hazard could be eliminated, the average production over a term of years could be increased by at least 25 and perhaps 50 per cent. For the last two seasons work has been in progress to test several substances that might prolong dormancy long enough to reduce the likelihood of damage from such frosts. The purpose of this paper is to report the results obtained in prolonging dormancy of tung buds by means of certain organic compounds.

Investigation

Healthy tung trees were used in an orchard near Floral City, Florida. Two to four representative branches, each having an average of thirty to sixty buds, were

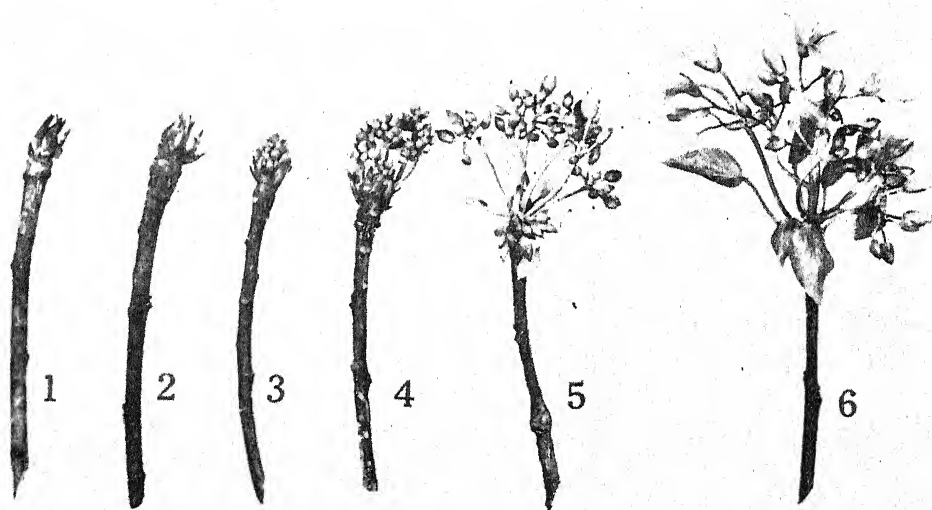


FIG. 1.—Specimens of tung buds illustrating degree of development of the first six classes

selected on each tree. Half of these were treated and the remainder used as controls. This branch unit method was adopted rather than a tree unit method because of the great variability in the blooming habit among individual seedling trees. Each treatment was applied to one branch on each of three different trees.

Three series of treatments were used, beginning February 10, February 25, and March 11, respectively. In each case repeat applications were made at intervals of 2 weeks until March 25. Thus four applications were required for the first series, three for the second, and only two for the series begun on March 11.

Three methods of application were used: (a) Direct injection (by means of a hypodermic syringe) of a 0.01 per cent aqueous solution of the potassium salt of alpha naphthalene acetic acid into the bud scale sheath formed around the flower primordia of dormant buds. (b) Application to the surface of the dormant buds

and twigs by means of a pressure sprayer, using two different spray formulas. In one case, a 0.01 per cent solution of alpha naphthalene acetic acid was prepared in a mixture containing 4 per cent dioxane and 1 per cent fish-oil soap solution; in the other mixture approximately 0.4 per cent by weight of summer spray oil was substituted for the fish-oil soap. The compounds indole-3-acetic acid and alpha naphthalene acetamide were substituted for the above substance in other spray treatments. In each case the organic substance was dissolved in the dioxane before mixing with the remainder of the ingredients. (c) Application of the organic substance in a lanolin emulsion to the surface of dormant buds by means of a small varnish brush. The emulsion was prepared according to the formula of HILDRETH and MITCHELL (4). However, 0.3 per cent solutions of alpha naphthalene acetamide and of indole-3-acetic acid were substituted for indole-3-butyric acid.

In order to assay the effectiveness of the treatment in prolonging dormancy, seven classes of buds were established on the basis of the degree of development. The midpoint of each class is typified by the specimens illustrated in figure 1. Class 7 (not illustrated in the figure) represents terminals on which petals have begun to fall. When the buds began to expand counts were made at intervals of 3-7 days to determine the comparative number of buds in each class on treated and control branches.

Of the three methods listed, only the last (c) produced any observable effect on dormancy of the buds. Whether the difference in the effectiveness of these methods is due to the difference in concentration of organic substances or to the manner of application has not been established. That the effect of prolonging dormancy was due to the organic substance rather than to the lanolin carrier was shown by field blank tests comparing buds treated with the lanolin carrier alone and untreated buds. No difference could be observed between these two groups. This was further confirmed by laboratory experiments with excised tung branches.

The data presented in table 1 summarize the results obtained with alpha naphthalene acetamide in lanolin emulsion. A similar trend was obtained with indole-3-acetic acid in the same carrier. Similar results were also obtained with excised tung branches brought into the laboratory, treated as outlined in (c), then placed in humid chambers with cut ends standing in about 2 inches of water.

The data indicate a pronounced effect of the treatment in delaying bud development. Those treatments in which applications were repeated three or four times were about equally effective in delaying bud development, but when the buds received only two applications, the effectiveness was slightly decreased. It is not yet possible to state whether the number of times the treatment was repeated or the delayed date of initial application was the more important factor in producing the decreased effectiveness of the treatments repeated only twice.

Preliminary experiments by FERNHOLZ and POTTER (8) indicate that the im-

TABLE 1
EFFECT OF ALPHA NAPHTHALENE ACETAMIDE IN LANOLIN EMULSIONS IN PROLONGING DORMANCY IN TUNG BUDS; 1941 SEASON

DATE OF FIRST APPLICATION	No. OF TIMES TREATMENT REPEATED	DATE OF BUD COUNT	STATE OF BUD DEVELOPMENT (DATA AS PERCENTAGES)														INDEX*	
			CLASS 1		CLASS 2		CLASS 3		CLASS 4		CLASS 5		CLASS 6		CLASS 7			
			TREAT-ED	CON-TROLS	TREAT-ED	CON-TROLS	TREAT-ED	CON-TROLS	TREAT-ED	CON-TROLS	TREAT-ED	CON-TROLS	TREAT-ED	CON-TROLS	TREAT-ED	CON-TROLS		
2/2/10.....	4	4/4	94.2	45.6	4.3	45.6	1.5	6.6	0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	107	165
2/2/10.....	4	4/8	78.7	5.4	13.2	13.5	5.9	20.8	2.2	53.7	0.0	6.6	0.0	0.0	0.0	0.0	132	343
2/2/10.....	4	4/11	28.4	0.4	31.4	6.3	33.6	24.2	4.4	18.8	2.2	36.8	0.0	13.5	0.0	0.0	221	426
2/2/10.....	4	4/16	14.0	0.5	3.7	0.0	2.2	0.5	63.9	15.0	6.6	7.3	1.5	58.9	8.1	17.8	382	577
2/2/10.....	4	4/23	8.4	0.0	1.7	0.0	3.4	0.0	0.0	0.0	7.6	0.0	31.0	1.9	47.9	98.1	581	698
2/2/25.....	3	4/4	89.7	36.3	9.3	41.4	1.0	21.1	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	111	187
2/2/25.....	3	4/8	82.0	5.4	6.6	13.5	10.4	20.8	1.0	53.7	0.0	6.6	0.0	0.0	0.0	0.0	130	343
2/2/25.....	3	4/11	57.3	0.4	16.7	6.3	10.4	24.2	7.3	18.8	7.3	36.8	1.0	13.5	0.0	0.0	194	426
2/2/25.....	3	4/16	29.8	0.5	3.2	0.0	8.5	0.5	40.4	15.0	2.1	7.3	12.8	58.9	3.2	17.8	333	577
2/2/25.....	3	4/23	24.5	0.0	0.0	0.0	0.0	0.0	4.3	0.0	8.5	0.0	13.8	1.9	48.9	98.1	509	698
3/3/11.....	2	4/4	61.9	25.2	28.6	36.4	7.5	38.4	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	150	213
3/3/11.....	2	4/8	42.6	0.6	10.5	3.1	20.3	13.6	26.6	75.3	0.0	7.4	0.0	0.0	0.0	0.0	231	386
3/3/11.....	2	4/11	23.8	0.6	17.3	1.9	25.2	6.2	20.1	27.8	12.2	45.6	1.4	17.9	0.0	0.0	284	470
3/3/11.....	2	4/16	18.9	0.0	1.4	0.0	1.4	0.0	36.3	6.9	2.1	4.4	23.8	72.5	16.1	16.2	437	598
3/3/11.....	2	4/23	8.9	0.0	0.0	0.0	3.4	0.0	0.7	0.0	6.9	0.0	21.2	0.0	58.9	100.0	596	700

* Obtained by summing the products of the percentage in each class and the class number; hence the smaller the index figure, the more retarded the bud development.

mature peduncles of tung flower buds are exceedingly susceptible to injury by low temperature. Thus when terminals in the tight cluster stage (class 3) are exposed to a temperature of 28°F ., very little injury occurs. When open clusters with the peduncles exposed (classes 4 and 5) are subjected to a temperature of 28°F ., all are either injured or killed.

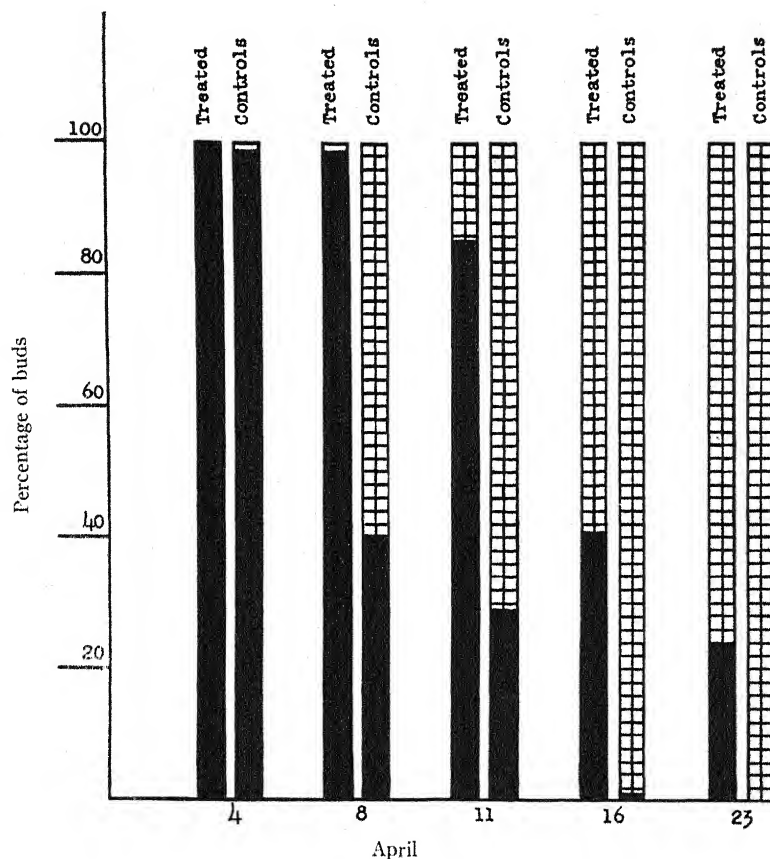


FIG. 2.—Degree of development of tung buds treated three times with alpha naphthalene acetamide as compared with untreated controls. Solid sections of columns represent classes 1, 2, and 3 (buds uninjured by temperature of 28°F .); open sections of columns represent classes 4, 5, 6, and 7 (buds seriously injured or killed by temperature of 28°F .).

These studies suggest that buds in class 4 or above will be damaged seriously by a light to medium frost, whereas it would take a heavy frost seriously to damage buds in classes 1, 2, and 3. In view of these considerations, the data in table 1 are expressed graphically in figure 2 to show the relative frost resistance to treated and control buds at the dates indicated on the graph. The more resistant buds in

classes 1, 2, and 3 were combined into one group and are represented by the solid portions of the bar graphs. The more expanded buds in the remaining less resistant classes are represented by the open portions. It may be noted that there was a period of about one week during which the injury to the treated branches by a frost of about 28° F. would probably have ranged between 0 and 15 per cent, whereas the injury to the untreated branches might have ranged from slight to 70 per cent. During the ensuing 12-day period the treated branches had from 24 to 40 per cent or more of buds not susceptible to injury by a light frost, while all or nearly all the buds on the controls would have been injured.

The data presented here are concerned merely with observations on prolonging dormancy of tung buds by means of certain organic compounds. No practical applications are suggested or recommendations intended. There is a possibility, however, that some practical applications may develop from future studies.

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LITERATURE CITED

1. GARDNER, F. E., MARTH, P. C., and BATJER, L. P., Spraying with plant growth substance for control of the pre-harvest drop of apples. *Proc. Amer. Soc. Hort. Sci.* 37:415-428. 1940.
2. GUTHRIE, J. P., Inducing "dormancy" in potato tubers with potassium naphthalene acetate and breaking it with ethylene chlorohydrin. *Science* 88:86. 1938.
3. ———, Inhibition of the growth of buds of potato tubers with the vapor of the methyl ester of naphthalene acetic acid. *Contr. Boyce Thompson Inst.* 10:325-328. 1939.
4. HILDRETH, A. C., and MITCHELL, J. W., Spraying is a new method of applying root promoting substances. *Florists Review* 84:13. 1939.
5. LINDNER, R. C., Effects of indoleacetic and naphthylacetic acids on development of buds and roots in horseradish. *BOT. GAZ.* 100:500-527. 1939.
6. LOEB, J., A quantitative method of ascertaining the mechanism of growth and of inhibition of growth of dormant buds. *Science* 45:436-439. 1917.
7. MITCHELL, J. W., and STEWART, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. *BOT. GAZ.* 101:410-427. 1939.
8. FERNHOLZ, D. L., and POTTER, G. F., Private communication.
9. THIMANN, K. V., and SKOOG, F., Inhibition of bud development and other growth substance in *Vicia faba*. *Proc. Roy. Soc. London B.* 114:317-319. 1934.
10. WINKELPLECK, R. L., Blossom delay with hormones. *Amer. Fruit Grower* 60:4. 1940.

EFFECTS OF STORAGE CONDITIONS ON CUT ROSES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 543

M. S. NEFF

Introduction

Successful storage of cut flowers should achieve several results: (a) The flowers should remain in the desired state of maturity or tightness of flower buds, during storage. (b) The flowers should retain their original color and color shadings or an equally satisfactory color, during storage. (c) It should be possible to store the flowers for a considerable period of time. (d) After removal from storage the flowers should retain their color and turgidity as long as would freshly cut flowers.

The rose, the most important flower in the florist industry, is one of the most difficult to store successfully. Under most conditions the cut buds develop characteristics that render them unsatisfactory when marketed in competition with those freshly cut. Various suggestions have been advanced toward a solution. PERRET (14) advocated the use of low temperatures and stressed the importance of the proper relative humidity during storage. HITCHCOCK and ZIMMERMAN (6) also demonstrated effects of these factors in relation to specific flowers. They stated that flowers stored at 5° C. for 7 days or longer may be expected to wilt much sooner when removed to a temperature above 20° C. than those freshly cut. NEFF and LOOMIS (13) and NEFF (11, 12) emphasized the importance of storing flowers with the flower stems out of water. THORNTON (19) found that suitable concentrations of carbon dioxide in the atmosphere of the storage container tended to prevent opening of rose buds during the storage period. Those stored in a carbon dioxide-enriched atmosphere lasted longer after removal to ordinary room conditions than similar ones that had been stored in an unenriched atmosphere. THORNTON considered 7 days the approximate limit of effective storage with carbon dioxide, because of petal drop.

The effect of storage conditions on the color of flowers has received considerable study. THORNTON (20) found that changes in the color of anthocyanin pigments of rose petals occurred when exposed to high concentrations of carbon dioxide. The bluing of the petals, that is, their tendency to become violet or purplish as they age, was correlated with changes in pH; the pH increased with the use of increased concentrations of carbon dioxide. In the case of Templar roses the loss of original color was correlated with a 0.27 increase in pH.

Change in color shading during storage is difficult to control. To overcome this

major difficulty it may be possible to stabilize the color in roses in such a way that storage conditions will not affect it. This may be accomplished eventually by treating natural coloring substances as dyes and stabilizing them in the petals, in somewhat the same manner that some dyes are stabilized. Most of the natural plant colors are mordant dyes (10, 15); that is, they must be adsorbed by a mordant or mordants if the color is to be stabilized. A mordant will adsorb a dye in the presence of a fiber (22) or combine with a dye (16) in forming insoluble colored compounds. Anthocyanin pigments, which constitute many of the colors of flowers, when mordanted with metallic salts tend to give dyeings fast to light but not to soap and water (15). Although theoretically any salt of a heavy metal is capable of acting as a mordant and adsorbing mordant dyes (10), in actual practice some salts are superior to others in this respect. Acid mordants include tannins, fatty acids, albumin, hydrous silica, arsenic acid, phosphoric acid, and various others. Metallic mordants are mainly hydrous oxides of the heavy metals, the most important of which are those of aluminum, chromium, copper, iron, and tin (22).

The color of a dye may be changed, depending on the mordant used. Alizarine red, for example, when dyed on a chrome mordant results in purplish red, on aluminum in bright red, on tin in scarlet, and on iron in a dull purple (10).

A clue to a possible role played by mordants in the living cell is suggested by the work of SHIBATA *et al.* (17). They state that organo-metallic complex compounds are formed between anthocyanin pigments and the salts of the alkaline earths and the heavy metals. They added metallic salts to solutions of certain extracted anthocyanin pigments. Such pigments extracted from *Rosa Gallica* turned red with HCl and acetic acid; violet red with CaCl_2 , SrCl_2 , MgCl_2 , and MnCl_2 ; light violet red with BaCl_2 and ZnCl_2 ; dark violet red with $\text{Al}_2(\text{SO}_4)_3$, Cr_2Cl_3 , and NiCl_2 ; red orange with $\text{Cu}(\text{C}_2\text{H}_3\text{O}_2)_2$ and HgCl_2 ; orange with NaOH and $(\text{NH}_4)_2\text{MoO}_4$; violet with SnCl_2 and Fe_2Cl_6 ; green with $\text{Pb}(\text{C}_3\text{H}_3\text{O}_2)_2$; yellow with K_2CO_3 . In other words, the range extended from green to deep red, depending on the salt used.

SHIBATA and coworkers are of the opinion that blue anthocyanin pigments in plants are complex organo-metallic compounds, the pigments probably being coordinated with calcium or magnesium. The violet, violet red, or red pigments are considered as analogous complex compounds containing fewer auxochrome hydroxyl groups or a mixture of the blue pigment complex and a red oxonium salt. The red oxonium salt is presumably formed with an excess of acid, the anion of the acid becoming attached to the pigment molecule.

CURREY (1) investigated bluing in roses. The variety Hadley was selected as the type which often blues and Lady Maureen Stewart as the one which seldom blues. He determined that the red color in the rose petals was produced by the

formation of a red oxonium salt between the pigment and the tannins and that the tannins were the only acids found in the cell sap. He noted that Lady Maureen Stewart contained 1.9 times more pigment and 2.0 times more tannin than Hadley. The former contained 1.39 times more tannin than pigment. The Hadley rose also contained 1.39 times more tannin than anthocyanin pigment. CURREY assumed that the tendency of Hadley to turn blue was due to the fact that it contained fewer tannins than the other rose. He disregarded the fact that Hadley also had a reduced amount of pigment directly proportional to the reduced amount of tannin. It would be just as logical to assume that bluing of Hadley is due to less pigment in the petals.

CURREY also considered that the ash analysis of the two roses showed no significant differences. By recalculating his figures, however, it is obvious that Lady Maureen Stewart had 0.9 as much silica as Hadley, 1.4 times more aluminum and iron, 1.3 times more lime, 1.1 times more magnesium, 0.8 as much sulphur, 1.3 times more phosphorus, 2.4 times more soda, and 1.3 times more potash. Aluminum and iron are both important mordant elements. Lady Maureen Stewart contained more of these elements. It is possible also that the lack of salts was a factor in the bluing of Hadley.

HALL (5) reports that in dyeing, tannic acid can unite with metallic salts to form insoluble compounds and that these have the power of retaining dyes. The compounds containing tannic acid, metal, and dyestuff are more nearly insoluble and stable than similar ones in which the metallic salts are absent. PERKIN and EVEREST (15) state that the great increase of the basicity of the oxygen atom of the pyrone ring that arises in passing from the flavonol pigment to the anthocyanin pigment is presumably the cause of the latter having the power of dyeing on tannins as well as basic mordants. It is possible that organo-metallic compounds that might be formed with anthocyanin pigments increase the molecular weight of the pigment and thereby influence color (4).

A number of investigators have attempted to maintain petal turgidity and brilliant color of cut flowers by placing the stems in various solutions. DUCOMET and FOURTON (2) found that the following aqueous solutions gave positive results: sugar and sodium chloride, sugar and dipotassium phosphate, sugar and chloral, sugar and manganese sulphate. LAURIE (9) indicated that combinations of hydrazene sulphate, copper sulphate, sucrose, sodium amytal, and metallic zinc powder in proper concentrations may reduce bacterial activity, increase transpiration, lower the rate of respiration, and provide the most suitable pH and osmotic concentration. KNUDSEN (8) and HITCHCOCK and ZIMMERMAN (6) placed the stems of cut flowers in aqueous solutions containing various chemicals and found no solution of any particular value. VOLZ (21) used a solution of aluminum sulphate in water.

There are available at present at least two commercial preparations¹ for use in aqueous solutions. Both are principally sugar and aluminum salts. The length of time during which cut roses remain turgid and of good color is considerably increased by the use of these solutions. After a few days the petals of red roses lose their original color, just as do those with the stems in water, but the former maintain highly satisfactory and pleasing color as contrasted with the dull violet or bluish tinges of the latter. The petals also maintain exceptional turgidity. There is apparently no clogging of the conducting tissues of the stems; at least it is never necessary to maintain petal turgidity by clipping off the ends of the stems. This indicates a rather unusual internal physiological condition. Physiologically, aluminum salts have been reported as increasing the permeability of protoplasm, thus making it more permeable to sugars, increasing the action of diastase, slowing down photosynthesis (3), causing protoplasm to set and harden (18), and inhibiting anthocyanin production (7).

Apparently the aluminum mordant salts and sugars complement each other. If either is eliminated from the commercially available solutions, color and turgidity are not so satisfactory. With these solutions the color of the developing inner petals is affected to a much greater extent than the matured outer ones, which tend to fade and blue during storage. The solutions would be more desirable if the color of the matured outer petals were preserved. An undesirable feature of storage of roses with the stems in water or an aqueous solution is the tendency of the buds to continue to open during storage.

NEFF (12) reported favorable results in regard to the preservation of color and keeping qualities of cut roses under conditions of light, low temperature, and the use of commercially available solutions and others containing potassium nitrate and sucrose.

Experimental results

Red Hollywood rose buds stored at 32°–38° F. for any length of time have a tendency to lose the original color shadings and begin to blue. Storage at low temperature and in carbon-dioxide concentrations high enough to cause the buds to remain in a tight condition resulted in considerable tendency to blue. This was also true for roses stored at low temperature in an atmosphere containing added carbon dioxide and reduced oxygen.

Generally the petals of roses previously stored expanded rapidly after removal from storage. The petals were crinkly along the edges and seemed to lack substance. The flowers failed to remain as turgid or as well colored as freshly cut roses under similar conditions.

¹ U.S. patents 2168304 and 2230931.

TURGOR AND LOW TEMPERATURE

The manner in which roses are handled previous to storage determines to a considerable degree their response under various conditions of storage. Fresh Hollywood roses that had never been placed in water were divided into groups of six each and exposed to the drying atmosphere of a room at 32°–38° F. for 3, 12, and 24 hours. After the drying treatment, the roses were placed under bell jars to protect them from further excessive loss of moisture. The stems were not in water at any time during the drying treatment or during the remaining storage period. An additional group of roses was left with the stems in water throughout the experiment.

The roses exposed for 3 hours lost 0.46 per cent, for 12 hours 0.7 per cent, and for 24 hours 2.7 per cent of their original weight. At the end of the 12-day storage period all roses stored with the stems out of water had lost an additional 1.7 per cent of their weight.

After 12 days in storage the flowers were removed to a room at 72° F., the stems placed in water and a comparison made with fresh roses. The roses were weighed when they were first brought into room temperature and after 12, 22, 31, and 43 hours. The fresh roses gained 9.5 per cent in weight in the first 12 hours at room temperature but lost weight by the twenty-second hour. By 38.5 hours they weighed 5.4 per cent less than the original weight. The roses exposed to 3 hours of drying gained 7.7 per cent in weight the first 12 hours, 8 per cent the next 19 hours, but by the forty-third hour weighed 1.2 per cent less than their original weight. The original weight refers to the weight of the roses when first placed in storage. The buds of the stored roses opened a little more rapidly than did the fresh buds. The individuals exposed to drying for 12 hours gained 4.1 per cent in weight the first 12 hours, 4.1 per cent the next 10 hours, 2.1 per cent the following 9 hours, but weighed 6 per cent less than the original weight by the forty-third hour. The buds opened a trifle more slowly than fresh ones. Those exposed to 24 hours of drying gained 0.6 per cent in weight the first 22 hours at room temperature. The following 9 hours they weighed 15.5 per cent less than their original weight. They opened much more slowly than the fresh buds and were only half opened at the time they wilted. Those stored with their stems in water lost weight at room temperature and the buds opened faster than fresh ones.

These results suggest turgor control as a method of maintaining rose buds in a tight condition during storage. Buds opened least during storage when exposed to the drying atmosphere for 24 hours before being placed under bell jars. Twenty-four hours of drying was apparently too long, as they failed to open properly when removed from storage and placed at room temperature with the stems in water.

None of the preceding lots retained their original color shadings during storage; all had a tendency to blue. When removed to room temperature none of the storage lots retained petal turgidity or color as well as did freshly cut roses.

LOW TEMPERATURE AND CARBON DIOXIDE

Rose buds were inclosed in large metal containers and placed in storage at 32°–38° F. The tops of the containers were made with grooves which were filled with water, into which the edge of the lids fitted. This arrangement provided a water seal, preventing excessive loss of carbon dioxide. A layer of water on the bottom of the containers maintained a reasonably high humidity. The buds were stored in the containers either with their stems in water or supported on wire racks out of water. The containers were fitted with two valves, through which carbon dioxide could be introduced. Carbon dioxide concentrations of 2, 5, 10, 15, and 20 per cent were maintained. After 12–20 days of storage the roses were inferior to freshly cut ones. The chief difficulty was their tendency to blue; the higher the carbon dioxide concentrations the greater was the tendency to do so. At concentrations of 2 and 5 per cent the buds did not remain as tightly closed as when higher concentrations were used. The roses stored with carbon dioxide were inferior to fresh ones when comparisons were made as to color and turgidity under ordinary room conditions.

Sweet peas were similarly stored with stems out of water at 32°–38° F. but without the use of carbon dioxide. High humidity in the individual chambers kept the petals fresh and turgid over a 16–21-day storage period. The specimens were removed from storage in excellent condition, with no petal drop. The stored flowers were turgid almost as long as fresh blooms when subsequently placed at room temperature with the stems in water, but they had lost all odor during storage.

CARBON DIOXIDE AND REDUCED OXYGEN

Talisman rose buds were stored as previously stated in metal containers at 32°–38° F. for 5 days in atmospheres consisting of 9–10 per cent carbon dioxide and varied amounts of oxygen. To obtain the desired atmosphere, air was displaced from the metal containers by means of nitrogen gas until the desired level of oxygen was secured, then carbon dioxide was introduced. At the end of 5 days the buds stored in 4.4 and 6 per cent oxygen had opened very slightly; in 9 per cent oxygen they had opened noticeably; and in 11 per cent oxygen still more so. At the end of 5 days the roses were unintentionally frozen. This injured the stems somewhat, particularly directly below the calyx cup. The samples in 11 per cent oxygen had the least stem injury; it was progressively greater in 9 and 6 per cent, up to 4.4 per cent oxygen, where the greatest injury occurred.

LOW TEMPERATURE, LIGHT, STEMS IN SOLUTIONS

Freshly cut buds of Hollywood roses were stored in three closed metal containers (A, B, and C) in a refrigerated room at 32° – 38° F. or in two closed glass humidity chambers (D and E). High relative humidity was maintained by the layer of water on the bottom of each container. A sheet of window glass loosely placed over the top of the metal containers, in place of the metal cover, allowed for the admission of light as desired.

The buds were divided into groups of four previous to being placed in the containers. Container A received eight groups, B three groups, C two groups, humidity chambers D and E four groups. The lower portions of the stems were immersed in flasks containing Floralife, a commercial product, to which sucrose had been added at the rate of 60 gm. to a quart of solution.

Roses in container A received approximately 48 foot candles of Mazda light for an 18-day period. Container B received approximately 20 foot candles for an 18-day period. Container C was covered for the first 12 days so as to exclude light; during the last 6 days the cover was removed and the roses exposed to 10 foot candles of Mazda light. Roses in D (glass humidity cases) were illuminated by a 20-watt daylight-type fluorescent lamp. The flowers received approximately 60 foot candles of light at the surface of the petals. Container E was illuminated by Mazda light which was first passed through an aqueous CuCl_2 filter 2.2 cm. in thickness (1 gm. of CuCl_2 to 1000 ml. of water), resulting in approximately 26 foot candles of light at the surface of the petals. Light was supplied to D and E for the first 6 days of storage, then discontinued until the twelfth day, at which time illumination was resumed.

Preliminary work had indicated that if roses whose stems were in the solutions were taken from the refrigeration room from time to time it might be beneficial. Consequently groups 2 and 6 in container A, groups 1 and 3 in container B, and group 2 in container C were placed in the 72° F. room for 30-minute periods on the second, third, fourth, fifth, seventh, and eighth days of storage. Groups 3 and 7 from container A were subjected to two 30-minute periods at 72° F. on the second, third, fourth, and fifth days and one 30-minute period on the seventh and eighth days of storage. The second day of storage, groups 4 and 8 of container A were placed at 72° F. for 30 minutes for six consecutive transfers. The third and fourth days they were taken out of the storage temperature twice a day for 30-minute periods; the seventh and eighth days they were removed only once a day. Group 1 of containers A, B, C, D, and E were not removed from the storage temperature until the end of the experiment.

Roses in D and E were transferred to the 72° F. room for 30-minute periods the second day of storage in the case of groups 2, 3, and 4. On the fourteenth day of storage group 2 in case D and group 2 in case E were placed in the 72° F. room

for 30 minutes, and then at 33° F. for 30 minutes for four consecutive transfers. Groups 3 in D and E were given six consecutive transfers. Group 4 was given eight consecutive transfers of 30 minutes each.

Table 1 shows the keeping qualities of the roses after removal from storage to a room at 72° F. with the stems in water.

TABLE 1
CONDITIONS OF STORAGE AND KEEPING QUALITIES OF HOLLYWOOD ROSES AFTER REMOVAL FROM STORAGE* TO ROOM AT 72° F., WITH STEMS IN WATER. STORAGE TEMPERATURE APPROXIMATELY 32°-38° F.; HUMIDITY HIGH

CON- TAINER	GROUP	DAYS IN STOR- AGE	HOURS WITH STEMS IN SOLU- TION	HOURS IN LIGHT		FOOT CANDLES OF LIGHT		TIMES TRANS- FERRED	NUMBER OF TURGID FLOWERS PER GROUP PER DAY AFTER REMOVAL FROM STORAGE TO ROOM AT 72° F. (STEMS IN WATER)									
				MAZ- DA	FLU- ORES- CENT	MAZ- DA	FLU- ORES- CENT		DAYS									
									1	2	3	4	5	6	7	8	9	
A	1....	8	192	192	0	48	0	0	4	4	4	2	
	2....	8	192	192	0	48	0	6	4	4	4		
	3....	8	192	192	0	48	0	10	4	4	4	3		
	4....	8	192	192	0	48	0	12	4	4	4	4		
	5....	18	432	432	0	48	0	0	4	4	1	1		
	6....	18	432	432	0	48	0	6	4	3	3	3	2	1		
	7....	18	432	432	0	48	0	10	4	4	4	4	4	2		
	8....	18	432	432	0	48	0	12	4	4	4	3	3		
B	1....	8	192	192	0	20	0	6	4	4	4	2		
	2....	18	432	432	0	20	0	0	4	4	4	4	4	3	2	...		
	3....	18	432	432	0	20	0	6	4	4	3	3	2	1		
C	1....	18	432	144	0	10	0	0	4	4	4	4	3	1		
	2....	18	432	144	0	10	0	6	4	4	4	2	1	1		
D	1....	14	336	0	192	0	60	0	4	4	4	4	3	2		
	2....	14	336	0	192	0	60	5	4	4	4	4	2	2		
	3....	14	336	0	192	0	60	7	4	4	4	4	4	3	2	...		
	4....	14	336	0	192	0	60	9	4	4	4	4	4	3	2	...		
E	1....	14	336	192†	0	20	†	0	4	4	4	4	3	2		
	2....	14	336	192†	0	20	†	0	5	4	4	4	3	3		
	3....	14	336	192†	0	20	†	0	7	4	4	4	4	4	2	1	1	
	4....	14	336	192†	0	20	†	0	9	4	4	4	4	4	3	1	...	

* Fresh buds with stems in water and under room conditions remained turgid 3-4 days.

† Filtered light.

As indicated by table 1, the storage roses may remain turgid longer than fresh ones at ordinary room temperature, and those stored 18 days may remain turgid longer than those stored 8 days. HITCHCOCK and ZIMMERMAN (6) stated that flowers stored at 5° C. for 7 days or longer may wilt much sooner when removed to temperatures above 22° C. than fresh flowers. It evidently takes a certain amount of time in storage before the effect of the conditions become permanent after the flowers are removed to room temperature and the stems placed in water. This permanent effect on turgidity of petals seems to be correlated with the state

of development of the buds. Rose buds tend to open, even under conditions of low temperature, especially if they are in water or a water solution. By the fifth to seventh day of storage this tendency becomes rather marked; up to that time the buds remain relatively tight. The roses removed after 8 days of storage were one-third or less open and so had not developed sufficiently for the storage treatment to have a permanent effect on the turgidity of the petals. On the other hand, at the end of the 18-day storage period the roses were one-half to two-thirds open, so were sufficiently advanced for the storage treatment to have a permanent effect on petal turgidity.

Table 1 indicates that removing roses to room temperature for short periods of time during storage may help to maintain petal turgidity after the flowers are finally removed and placed under ordinary room conditions with the stems in water. This may be true for roses stored 18 days and receiving 48 foot candles of Mazda light, or filtered Mazda light of 20 foot candles, but not for roses receiving 48 foot candles of Mazda light for only 8 days or 20 foot candles for 18 days.

One of the effects of shifts in temperature during storage is the appearance of drops of sweet liquid which exude from stems and the veins of the leaves. Another effect is the appearance of brown spots on the petals, apparently caused by the high concentration of sugar. The roses exposed to fluorescent light showed considerably less exudation than those stored under other types of light. At the time of removal from storage there were no brown spots on the petals, when it was used. After a few hours at room temperature, however, the brown spots appeared. Another result from the use of fluorescent light is that the petals opened in a normal manner after removal to room temperature. After storage under Mazda light the petals were crinkly and definitely not normal in their subsequent development at room temperature.

SOLUTIONS

Several mixtures were developed that were successful when used in solution in maintaining petal color and petal turgidity. The solutions are basically water, sucrose, and various metallic mordants. Tables 2 and 3 list the proportions of salts and sucrose used per 50 ml. of tap water, a solution sufficient for one rose. Greatly increasing the amount of solution used per rose may result in excessive injury to the stems or petals. Better Times roses were used in these experiments.

The following sugar solutions with single salts added produced indifferent results: lead acetate, zinc sulphate, aluminum acetate, aluminum chloride, aluminum oxide, ferrous oxalate, ferrous phosphate, lead chromate, and ferrous ammonium sulphate. A sugar solution with potassium dichromate and oxalic acid and a solution composed of sugar with tannic acid and acetic acid gave only fair results.

The following sugar solutions with two salts were used with varied results. A stock solution sufficient for one rose was made up of 0.02 gm. of ferrous sulphate, 1.25 gm. of sucrose, and 50 ml. of tap water. To this basic solution was added 0.02 gm. of one of the following salts: cadmium chloride, silver sulphate, cobalt

TABLE 2
EFFECT OF SOLUTIONS* ON COMPARATIVE KEEPING QUALITIES OF FRESHLY
CUT BETTER TIMES ROSES UNDER ROOM CONDITIONS; 1.5 GM. SUGAR
ADDED PER ROSE

SALT PER ROSE	FERROUS CHLORIDE		FERROUS SULPHATE		CHROMIUM SULPHATE		CERIC SULPHATE		STANNOUS CHLORIDE		STANNOUS SULPHATE	
	PETALS TURGID† (DAYS)	ORIGI- NAL PH	PETALS TURGID (DAYS)	ORIGI- NAL PH	PETALS TURGID (DAYS)	ORIGI- NAL PH	PETALS TURGID (DAYS)	ORIGI- NAL PH	PETALS TURGID (DAYS)	ORIGI- NAL PH	PETALS TURGID (DAYS)	ORIGI- NAL PH
0.015	4	5.6	6	5.4	4	4.6	5	3.9
0.02	6	4.9	6	5.5	6	4.5	5-6	3.5
0.025	5	4.7	6-7	5.1	4	4.2	5-6	3.3	6	2.8
0.03	6	5.0	4	4.0	5-6	3.0
0.035	6	4.8	4	3.8	5-6	2.9

* Similar buds with stems in water and under room conditions remained turgid 3-4 days.

† Or until they dropped.

TABLE 3
EFFECT OF SOLUTIONS* ON COMPARATIVE KEEPING QUALITIES OF
FRESHLY CUT BETTER TIMES ROSES

SALT USED	SUGAR PER ROSE (GM.)	SALT PER ROSE (GM.)	ORIGI- NAL PH	PET- ALS TURGID (DAYS)	PH OF SOLUTIONS DETERMINED TWICE DAILY									
					SECOND DAY		THIRD DAY		FOURTH DAY		FIFTH DAY		SIXTH DAY	
					A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
Ferrous chloride. . .	1.25	0.02	5.2	6	5.2	4.7	4.3	4.1†	4.9	4.7	4.7	4.8	4.7	4.7
Ferrous sulphate. . .	1.25	0.03	5.0	5-6	4.8	4.3	4.3	3.9†	4.9	4.4	4.6	4.6	4.5	4.4
Ferrous chloride. . .	0.125	0.02	5.2	6	5.0	4.5	4.1	3.8†	5.2	4.9	5.0	5.1	5.0	4.9
Ferrous sulphate. . .	0.125	0.03	5.1	5	5.2	4.6	4.2	4.0†	5.0	4.7	4.8	5.0	4.7	4.6
Ferrous chloride. . .	0.25	0.02	5.2	6-7	5.4	5.1	4.5	4.2†	4.6	4.5	4.5	4.5	4.7	4.7
Ferrous sulphate. . .	0.25	0.03	5.1	6-7	5.3	4.7	4.4	4.2†	5.0	4.7	4.8	4.9	4.7	4.6

* Fresh buds with stems in water and under room conditions remained turgid 3-4 days.

† Tap water added to each container, replacing water lost through evaporation and transpiration.

sulphate, cobalt chloride, sodium tungstate, uranium chloride, antimony chloride, antimony sulphate, bismuth acetate, molybdic acid, copper sulphate, ferric chloride, nickel chloride, nickel sulphate, lead chloride, manganese chloride, and mercurous chloride. Most of the solutions proved too concentrated, as shown by the

excessive burning of the stem or wilting of the petals. The use of molybdc acid, cobalt, bismuth, lead, uranium, and tin salts appeared to have promise.

Summary

1. Rose buds may be maintained in the desired state of maturity during storage at low temperature by avoiding placing the stems in water or aqueous solution during such period. The desired state of maturity also may be maintained by storing the roses in an atmosphere containing added carbon dioxide, or carbon dioxide and a reduced amount of oxygen.
2. Rose buds have a strong tendency to open during storage. This is particularly true when stored with their stems in water or aqueous solutions.
3. Roses develop objectionable color characteristics under low temperature storage. These tendencies seem to increase when stored in an atmosphere containing large amounts of carbon dioxide, or carbon dioxide and a reduced amount of oxygen.
4. As a rule, roses which have been stored do not maintain satisfactory petal turgidity or petal color after removal from storage to room temperature with their stems in water.
5. Roses which have been stored with the stems in a sugar-mordant solution under proper conditions of light and temperature and for a sufficient length of time may remain turgid longer than fresh roses when placed in water at room temperature.
6. Solutions containing water, sucrose, and a salt of certain heavy metals may materially aid in maintaining petal color and turgidity of cut roses.

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LITERATURE CITED

1. CURREY, G. S., The cause of blueing in red roses. *Jour. Roy. Soc. New So. Wales* 61:307-314. 1927.
2. DUCOMET, V., and FOURTON, L., Preservation of cut flowers. *Florists Exch.* 31:597-598. 1911.
3. FLURI, M., Der einfluss von Aluminumsalzen auf das Protoplasma. *Flora* 99:81-126. 1909.
4. GEORGIEVICS, G. VON, and GRANDMOUGIN, E., The text-book of dye chemistry. Transl. and revised from 4th German ed. by A. Mason. London. 1920.
5. HALL, A. J., Dyes and their application to textile fabrics. London. 1923.
6. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Effects of chemicals, temperature, and humidity on the lasting of cut flowers. *Contr. Boyce Thompson Inst.* 2:196-203. 1930.
7. KATIC, D. L., Beitrag zur Kenntniss der Bildung des roten Farbstoffs in vegetativen Organen der Phanerogamen. Inaugural-Dissertation Halle a.S., 1905. (Cited from WHELDALE, M., The anthocyanin pigments of plants. 1916.)
8. KNUDSEN, L., Preserving cut flowers. *Amer. Flor.* 43:649-650. 1914.

9. LAURIE, A., Studies of the keeping qualities of cut flowers. *Amer. Soc. Hort. Sci.* 33:595-597. 1936.
10. MATTHEWS, J., Application of dyestuffs. New York. 1920.
11. NEFF, M. S., Problems in the storage of cut carnations. *Plant Physiol.* 14:271-284. 1939.
12. ———, Color and keeping qualities of cut flowers. *BOT. GAZ.* 101:501-504. 1939.
13. NEFF, M. S., and LOOMIS, W. E., Storage of French marigolds. *Proc. Amer. Soc. Hort. Sci.* 33:638-685. 1936.
14. PERRET, A., Le froid en horticulture. *Rev. Sci. Paris* 5:170-174. 1907.
15. PERKIN, A. G., and EVEREST, A. E., Natural colouring matters. London. 1918.
16. PERKIN, W. H., and KIPPING, F. S., Organic chemistry. Philadelphia. 1911.
17. SHIBATA, A. G., SHIBATA, Y., and KASIWAGI, I., Studies on anthocyanins: Color variations in anthocyanin. *Jour. Amer. Chem. Soc.* 41:208-220. 1919.
18. SZÜCS, J., Über einnige charakteristische Wirkungen des Aluminiumions auf das Protoplasma. *Jahrb. wiss. Bot.* 52:269-332. 1913.
19. THORNTON, N. C., The use of carbon dioxide for prolonging the life of cut flowers, with special reference to roses. *Contr. Boyce Thompson Inst.* 2:535-547. 1930.
20. ———, Changes in flower color as evidence of the effectiveness of carbon dioxide in reducing the acidity of plant tissue. *Contr. Boyce Thompson Inst.* 6:403-405. 1934.
21. VOLZ, E. C., Studies on the keeping qualities of cut flowers. Unpublished thesis, Cornell University. 1918.
22. WEISER, H. B., Inorganic colloid chemistry. Vol. II. The hydrous oxides and hydroxides. New York. 1935.

AUXIN STORAGE AS RELATED TO ENDOSPERM TYPE IN MAIZE

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The extent to which auxin storage is related to kind of tissue is as yet unknown. Maize endosperms provide an excellent source of material to investigate this point; first, because a method exists (1) for the total extraction of auxin from them, and second, because they provide ontogenetically identical tissues which may differ genetically, the latter leading to the storage of different kinds and proportions of materials. Five well-known endosperm types were chosen for this investigation: brittle, waxy, floury, sugary, and two kinds of flint.

The first extraction method used involved successive washes with water. Embryos and endosperms were carefully separated, and the latter finely ground in a glass mortar. A 1-gm. sample of this ground material was placed on an asbestos filter and given twenty successive washes, each with 10 cc. of water (at 2-minute intervals). The aqueous filtrates from these twenty washes were collected in one flask; these and all subsequent samples were assayed by the deseeded *Avena* method. The twenty-first wash was collected and assayed separately in order to determine whether most of the auxin had been removed in the original washes. In no instance did the twenty-first wash contain as much as half of 1 per cent of the auxin extractable by this method. This experiment was repeated six times for each endosperm type, with good agreement, and the averages of these data are presented in table 1 (method A).

After these experiments were completed, an extraction method was developed (1) which makes it possible to distinguish between the relative amounts of free auxin and auxin precursor present in a given sample of maize endosperm. The so-called precursor is an as-yet unidentified compound which upon alkaline hydrolysis yields auxin.

For determining the free auxin content of the different endosperm types by the newer method, ground tissue was prepared as described, and 0.25-gm. samples were allowed to stand in 25 cc. of distilled water at 25° C. for 10 minutes. This preparation was centrifuged and the clear extract diluted to suitable concentrations for obtaining proportionality curvatures in the assay. The yields of free auxin (method B) from the different endosperm types are also presented in table 1.

Determinations of total auxin were made by heating 0.25-gm. samples of ground endosperm in borate buffer at pH 9.6 and 120° C. for 15 minutes. The hydrolyzed samples were neutralized, centrifuged, and dilutions made up for assay. The total

auxin yields thus obtained are included in table 1 (method C). The auxin precursor consists of the total auxin present after hydrolysis, minus the free auxin.

It may be noted from the table that the yields as determined by method B agree in a general way with those obtained by method A, that is, both methods give free auxin, but method B is much simpler. The free auxin content is of the same order for brittle, waxy, floury, and Canada flint, twice as high for sugary, and half as high for pop corn. Auxin precursor yield is from six to sixteen times that of the free auxin. It is of about the same magnitude in brittle, waxy, floury, and Canada

TABLE 1
AUXIN YIELDS FROM DORMANT MAIZE ENDOSPERMS OF
DIFFERENT TYPES (EMBRYOS REMOVED)

ENDOSPERM TYPE*	AUXIN YIELDS IN MILLIONS OF TDC PER GRAM DRY WEIGHT (OR TENS OF MICROGRAMS OF INDOLEACETIC ACID)			
	METHOD A	METHOD B	METHOD C	AUXIN PRECURSOR (TOTAL AUXIN MINUS FREE AUXIN)
	FREE AUXIN (21 WASHES WITH WATER)	FREE AUXIN (1 WATER EXTRACTION, 10 MIN. AT 25° C.)	TOTAL AUXIN (WATER SUS- PENSION AUTO- CLAVED AT PH 9.6 AND 120° C. FOR 15 MIN.)	
Brittle.....	0.56	0.71	5.2	4.5
Waxy.....	0.64	0.45	5.9	5.4
Floury.....	0.54	0.58	4.5	3.9
Sugary.....	1.5	1.3	13.2	11.9
Flint (pop corn).....	0.26	0.20	3.2	3.0
Flint (Canada flint).....	0.45	0.50	6.0	5.5

* First four samples obtained through the courtesy of the Department of Plant Breeding, N.Y. State College of Agriculture, Cornell University.

flint. In common with free auxin, it is somewhat lower in pop corn endosperm and markedly higher in sugary.

Thus of all the endosperm types tested, sugar corn endosperm was found to be the richest in both auxin and auxin precursor.

Auxin assays of four commercial varieties of sugar corn gave the results reported in table 2. Of these, the variety Country Gentleman possessed the highest total auxin (free auxin plus auxin precursor).

Auxin assays on immature corn kernels in various stages of development showed young kernels much richer in auxin than mature dormant ones. Such immature kernels are available at all times in commercial, dry-pack canned and frozen corn. Tests were therefore made on "Niblets" (Minnesota Valley Canning Company) and frozen cut Golden Bantam corn (Frosted Foods). Ten endosperms from each

sample were ground, heated in borate buffer at 120° C. and pH 9.6 for 15 minutes, neutralized, centrifuged, and the clear extract diluted for assay. Total auxin content of the Niblet endosperms was 32 million TDC per gram dry weight (equivalent to 320 micrograms of indoleacetic acid) and of the frozen endosperms 34 million TDC per gram. The former had more than 3 million TDC free auxin per gram dry weight, the latter only 0.9 million.

In view of the facts that indoleacetic acid has been isolated from corn (2) and that the auxin derived from the precursor is alkali stable (1), it is probable that 90 per cent or more of the auxin obtainable from corn is indoleacetic acid. The total auxin yields reported for dry-pack canned and frozen corn (immature stages of

TABLE 2

TOTAL AUXIN PRESENT IN DORMANT KERNELS
OF FOUR VARIETIES OF SUGAR CORN*

VARIETY	AUXIN†
Bantam Evergreen no. 36010	9.0
Stowell's Evergreen no. 26172	11.4
Country Gentlemen no. 38666	14.2
Early Evergreen no. 26045	8.4

* Samples obtained through the courtesy of the Associated Seed Growers, Inc., Milford, Connecticut.

† In millions of TDC per gram dry weight of entire kernels (or in tens of micrograms of indoleacetic acid).

sugar corn) are the equivalent of more than 300 mg. of indoleacetic acid per kilogram dry weight.

It is clear that kernels of sugar corn are much richer in auxin than those with brittle, waxy, floury, or flint endosperm. So far as can be determined, these are the first assays demonstrating markedly different auxin storage in ontogenetically identical tissues from different varieties of the same species.

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LITERATURE CITED

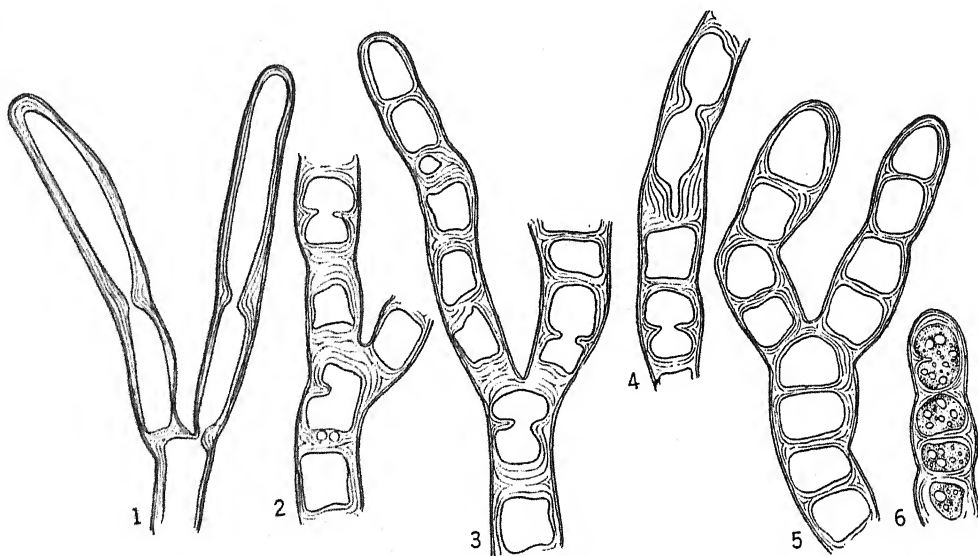
1. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., The total extraction of free auxin and auxin precursor from plant tissue. *Amer. Jour. Bot.* 28:596-607. 1941.
2. HAAGEN SMIT, A. J., LEECH, W. D., and BERGREN, W. R., Estimation, isolation and identification of auxins in plant material. *Science* 93:624-625. 1941.

AKINETE FORMATION IN VAUCHERIA GEMINATA

M. S. RANDHAWA

(WITH EIGHT FIGURES)

An interesting variety of *Vaucheria geminata* (Vauch.) DC, probably var. *longistipitata* Chapman 1934, was collected by the writer in December, 1939, from the Chenab Gardens, Chak no. 45 G.B., near Gojra, District Lyallpur, Punjab, growing in the shade by the side of a water course. On examination it was found to be so extensively segmented that it hardly appeared like a *Vaucheria* at all. This



FIGS. 1-6.—Fig. 1, primary septation into coenocytes. Figs. 2-4, advanced stages in akinete formation. Fig. 5, mature akinetes. Fig. 6, same showing contents.

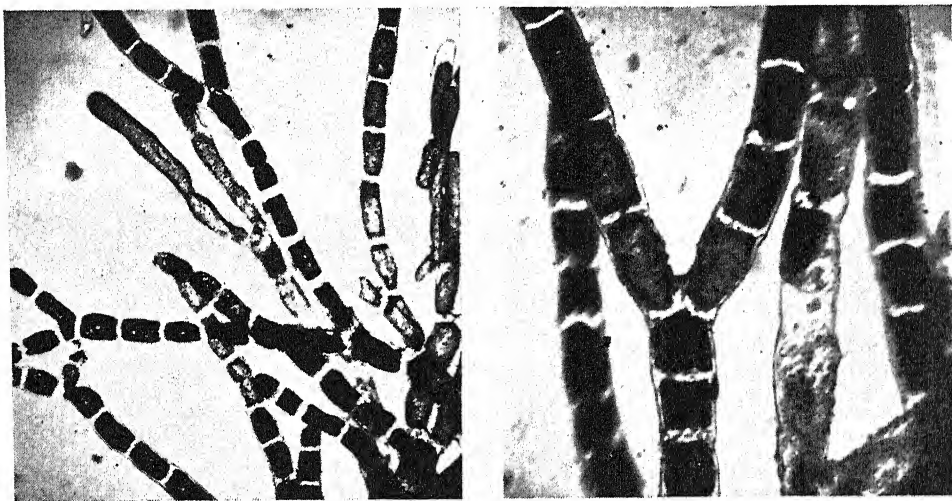
alga was again collected in December, 1940, and the same extensive akinete formation was found as in the previous year.

The purely vegetative filaments are 30-51 μ in diameter and display extensive dichotomous branching, the branching being radially arranged. Sex organs are very rarely formed and are borne at the ends of comparatively long branches, as in CHAPMAN'S *longistipitata* variety of *V. geminata* described from Urbana, Ohio (1).

The interest of this form lies in its extensive akinete formation. This mode of propagation is so common in this alga that it has practically displaced the sexual

mode of propagation, which is found only as a relic sufficient to establish its identity.

A primary septation of the filament takes place by the thickening of the cell wall, on account of the apposition of mucilaginous material at particular points on both sides (fig. 1). These thickening bands are deposited radially, and protoplasm on both sides of the septa connects by means of narrow openings (fig. 2). This condition has a superficial resemblance to that sometimes obtained in *Sphaeroplea africana* Fritsch, where, however, the septa are composed of a number of radial processes, while in this alga they are comparatively homogeneous. The septa grow



FIGS. 7, 8.—Fig. 7 (left), general habit and early stages in akinete formation. Fig. 8 (right), parts of filaments showing akinetes.

radially and centripetally, ultimately meeting in the middle and fusing. Thus the filaments segment into a number of coenocytes, as in *Cladophora glomerata*. This process of the ingrowth of the cell wall is repeated until the coenocyte is subdivided into a number of akinetes (figs. 5, 8). Coenocytes constricted in the middle, somewhat dumbbell-shaped, may commonly be found (figs. 2-4). Sometimes ingrowth of cell wall may take place from one side only (figs. 2, 6). In this process of segmentation, islands of protoplasm may be left surrounded by thick mucilaginous material of the septa (fig. 3). Narrow projections of protoplasm, connected with the parent coenocyte on one side and recently cut off from a daughter akinete, may also be seen (fig. 4). Mature akinetes, more or less rectangular in outline, are rich in oil content and other food reserves, and sometimes have a more or less frothy appearance. They are $15-33\ \mu$ long and $30-50\ \mu$ broad. The contents stain

deep blue with Nile blue. It has not been possible to trace the ultimate fate of the akinetes and the mode of their liberation.

Akinete formation in *Vaucheria geminata* has been described by STAHL (3). His figures show the so-called cysts widely separated by more or less transparent septa, which is hardly the case with the Indian form. Like STAHL's alga, here also septation was noticed in a terrestrial alga, which was exposed to the risk of complete desiccation in a short time on account of the periodic closing of the canal water supply in the water course. The thick mucilaginous walls insure the alga against the risk of sudden desiccation, and the septation provides a more prolific and efficient method of propagation, almost completely substituting for a comparatively complicated mode of sexual propagation.

As already observed, the dichotomously divided, richly segmented, and radially arranged branches of this alga hardly appear like those of a *Vaucheria* (fig. 7). In its general appearance and mode of septation, resemblances to *Cladophora* are obvious. That the coenocytic condition of Siphonales is derived from a septate condition appears plausible. The reappearance of a septate condition under unfavorable environmental conditions may be regarded as an atavistic feature. As in *Cladophora*, the formation of septa in this alga is not related in any way with nuclear division. OLTMANN's (2) view that the Siphonales, particularly the *Vaucheriaceae*, are derived from septate forms like *Cladophora* appears to be considerably strengthened from these observations on the mode of septation in this alga.

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LITERATURE CITED

1. CHAPMAN, F. B., The algae of the Urbana, Ohio, raised bog. Ohio Jour. Sci. 34:327-332. 1934.
2. OLTMANN, F., Morphologie und Biologie der Algen. I. 2d ed. Jena. 1922.
3. STAHL, E., Ueber die Ruhezustände der *Vaucheria geminata*. Bot. Zeitschr. 37:129-137. 1879.

CURRENT LITERATURE

Biological Symposia. Vol. VI. Edited by JACQUES CATTELL. Lancaster, Pa.: Jaques Cattell Press, 1942. Pp. xii+355. Illustrated.

This sixth volume in the series of biological symposia is under the editorship of TH. DOBZHANSKY. The subject matter is presented in three sections.

I. *Symposium on temperature*: Introduction by H. H. PLOUGH; Temperature and spontaneous mutation, by H. H. PLOUGH; Induction of polyploidy in animals by extremes of temperature, by G. FANKHAUSER; Temperature and the differentiation of characters in *Drosophila*, by G. P. CHILD; Temperature factors in the development and evolution of sex, by EMIL WITSCHI; Isolating mechanisms, evolution, and temperature, by H. J. MULLER; Form and function in frizzle fowl: the interaction of hereditary potentialities and environmental temperature, by WALTER LANDAUER; Seasonal factors in gall wasp distribution, by A. C. KINSEY; and Role of temperature in the speciation of frogs, by J. A. MOORE.

II. *Symposium on isolating mechanisms*: Role of isolation in the differentiation of plant species, by G. L. STEBBINS, JR.; Isolating mechanisms in a complex of four toad species, by A. P. BLAIR; Isolating mechanisms in gall wasps, by A. C. KINSEY; and Isolating mechanisms in the genus *Drosophila*, by J. T. PATTERSON.

III. *Symposium on genetic control of embryonic development*: Role of genetic differentials in the embryonic development of amphibia, by V. C. TWITTY; Morphogenesis of genetic abnormalities in the chick, by V. HAMBURGER; and Physiological genetics of melanin pigmentation of the guinea pig, by SEWELL WRIGHT.

The papers show an increasing realization among biologists that, no matter with what material and with the aid of what tools they may work, they are seeking an understanding and a control of life processes. These symposia are contributing greatly toward the attainment of these aims.—J. M. BEAL.

A Symposium on Respiratory Enzymes. Madison: University of Wisconsin Press, 1942. Pp. xii+281. \$3.00.

The book contains the addresses given at the symposium held co-operatively by the University of Wisconsin and the University of Chicago in September, 1941. The subjects discussed are intermediate carbohydrate metabolism; oxidative mechanisms in animal tissues; hydrogen transport; Pasteur effect; oxidases, peroxidases, and catalase; nicotinamide nucleotide enzymes; flavoproteins; cytochromes; phosphorylation of carbohydrates; metabolic cycles and decarboxylation; transamination; tumor, bacterial, and animal tissue respiration.

The book is an excellent summary of what is probably the most active phase of enzyme chemistry. No attempt is made to treat each enzyme exhaustively. Rather, the plan is to give recent developments, to interpret these in relation to past findings, and to call attention to unsolved problems. The structure, mechanism of the action, and properties of the enzymes are emphasized. An interesting feature is several pages of informal photographs of those attending the symposium, including a number of the participants.—S. V. EATON.

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